AD_____

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TITLE: Continuation of a Postdoctoral Research Associateship Program

PRINCIPAL INVESTIGATOR: Judith K. Nyquist, Ph.D.

CONTRACTING ORGANIZATION: National Academy of Sciences
Washington, DC 20418

REPORT DATE: December 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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FOREWORD

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____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Judith K. Nyquist

Judith K. Nyquist, Ph.D. October 16, 1997

PI - Signature Date

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NATIONAL RESEARCH COUNCIL

Resident Research Associateship Program

with the

U.S. Army Medical Research and Materiel Command

Status Report

October 1, 1996 to September 30, 1997

AMRDC DAMD 17-92-V-2002

PUBLICITY

The NRC Research Associateship Programs for the reporting period were announced to the scientific community in the Fall of the preceding year, 1995. Publicity materials describing the NRC-AMRMC Program were distributed in November to presidents, graduate deans, and heads of appropriate science and engineering departments of all academic degree-granting institutions in the United States. These materials were also sent to Program Representatives and Associateship Advisers at the participating laboratories and to other interested persons.

REQUESTS

Application materials were distributed in response to specific requests for information about the NRC-AMRMC Associateship Program or as a result of general requests by persons whose fields of specialization appeared to be appropriate for the research opportunities available in the AMRMC laboratories.

COMPETITION

Panel reviews of applicants for the Associateship Programs, including those with the U.S. Army Medical Research and Materiel Command, are conducted in February, June, and October of each year. The following is a breakdown of the action taken with the applications during the period of the report.

	Oct 96	Feb 97	Jun 97
Total Applications	10	10	8
Actions taken upon the above applications:			
Incomplete Documentation	1	1	-
Not Approve/Not Reviewed	3	6	-
Laboratory Rejection	1	-	-
Number of Applications Reviewed	5	3	8
Actions taken upon the reviewed applications:			
Non-Recommended	-	-	1
Recommended	5	3	7
Actions taken upon Recommended Applications:			
Accepted Award	5	2	7
Recommended/No Action	-	-	-
Recommended/No Funding	-	-	-
Alternate	-	-	-
Alternate with Final Turndown			
Withdrew after Review/Recommended	-	-	-
Declined award	-	1	-

ASSOCIATES' ACTIVITIES

Associates who ended tenure during the report period were on tenure for an average of 27 months, with a high of 39 months and a low of 8 months. In their termination reports, the associates indicated their amount of scholarly activity while on tenure as an associate. Associates reported the following activities:

39	Domestic Presentations	12	International Presentations
17	Published Articles in Refereed Journals	-	Patents applied for

After completing their tenure associates, indicated their future plans as follows:

-	College or University Professor	5	Industry
-	Student	-	Self-employed
3	National government, US or Foreign	-	Unemployed
-	Federal, State or Local Government	3	Another Post-Doctorate
-	Non-Profit	5	Other/No Information Provided

Associates on tenure as of October 1, 1996 are citizens of the following countries:

Argentina	1	Nigeria	2
England, U.K.	2	People's Republic of China	7
Ethiopia	1	Republic of Korea	2
France	1	Russia	1
India	7	United States	23
Israel	2	West Germany	1
Lativa	1		

Other information about the associates activities can be found in the following attachments and appendix.

Attachment 1 is a list of Associates who terminated their appointments during the period of October 1, 1996 through September 30, 1997. It includes the Associates' labs, their starting and termination dates, and the names of their Advisers. Associates are required to submit reports upon termination (attached to this report), and Advisers are asked to submit a final evaluation of each Associate. Associates who have not submitted a termination report have received a follow-up letter.

Attachment 2 provides a roster of Associates on Tenure as of October 1, 1997. This listing includes the Associate's Adviser, Division, start and expected termination date, and country of citizenship.

Attachment 3 lists the applicants who received awards during the period of October 1, 1996 through September 30, 1997. It includes the title of their Research Proposals.

Attachment 4 provides a roster of all recommended candidates by category (i.e. accepted, no funding, etc.). This report includes information about the recommended candidate's education, proposed research, starting date and adviser.

Attachment 5 details a cross tabulation of how many Associates were on tenure for the year in by center for each quarter within the report period and other yearly periods.

The *Appendix* contains the copies of the "Termination Reports" received from terminating associates.

Associates Who Ended Tenure 10/1/96 - 9/30/97 Attachment 1

AMRMC \ Medical Res Inst of Chemical Defense 10/17/97 [redacted]

Associate Name + Adviser		Division	Tenure Dates Start	End	Termination Report*	Adviser Report*
Korte, William D Dr. Ming L Shih	(S)	Pharmacology Division	10/01/96	7/31/97	Received	Not Recd

1 Associates Listed

+ (S) indicates the associate was a Senior.

Associates Who Ended Tenure 10/1/96 - 9/30/97 Attachment 1

AMRMC \ Medical Research Institute for Infectious Diseases 10/17/97 [redacted]

Associate Name + Adviser		Division	Tenure Dates Start	End	Termination Report*	Adviser Report*
Bray, Michael Peter Dr. John W Huggins	(S)	Virology Division	5/15/95	5/14/97	Received	Received
Canziani, Gabriela A Dr. Robert G Ulrich	(S)	Toxinology Division	2/04/94	1/21/97	Received	Not Recd
Connolly, Brett Michael Dr. Peter B Jahrling		Virology Division	10/18/93	10/17/96	Received	Not Recd
Gilligan, Kevin James Dr. Kevin Anderson		Divison not specified	7/18/94	5/30/97	Received	Received
Hevey, Michael Carl Dr. Alan L Schmaljohn		Virology Division	7/25/94	5/30/97	Received	Not Recd
Muldoon, Daniel F Dr. Robert B Wellner		Toxinology Division	6/05/95	6/03/97	Received	Not Recd
Woody, Mary Alice Dr. Bradley G Stiles		Toxinology Division	9/21/94	10/20/96	Received	Received

7 Associates Listed

+ (S) indicates the associate was a Senior.

Associates Who Ended Tenure 10/1/96 - 9/30/97 Attachment 1

AMRMC \ Research Institute of Medical Sciences 10/17/97 [redacted]

Associate Name + Adviser	Division	Tenure Dates Start	End	Termination Report*	Adviser Report*
Stewart, V Ann Dr. D G Heppner, Jr	Divison not specified	1/03/95	2/28/97	Received	Received

1 Associates Listed

+ (S) indicates the associate was a Senior.

Associates Who Ended Tenure 10/1/96 - 9/30/97 Attachment 1

AMRMC \ U.S. Army Research Institute of Environmental Medicine 10/17/97 [redacted]

Associate Name + Adviser		Division	Tenure Dates Start	End	Termination Report*	Adviser Report*
Lee, Dae Taek Dr. Kent B Pandolf		Divison not specified	9/15/94	11/15/96	Received	Received
Lewis, Steven Fred Dr. Harris R Lieberman	(S)	Divison not specified	5/16/96	9/15/97	Received	Not Recd
Shitzer, Avraham Dr. Richard R Gonzalez	(S)	Divison not specified	8/12/96	9/30/97	Not Recd	Not Recd
Wyatt, James Kelley Dr. Harris R Lieberman		Divison not specified	7/01/96	6/30/97	Received	Not Recd

4 Associates Listed

+ (S) indicates the associate was a Senior.

Associates Who Ended Tenure 10/1/96 - 9/30/97 Attachment 1

AMRMC \ Walter Reed Army Institute of Research 10/17/97 [redacted]

Associate Name + Adviser		Division	Tenure Dates Start	End	Termination Report*	Adviser Report*
Agin, Tonia Sue Dr. Marcia K Wolf		Division Of Medicine	2/14/94	2/13/97	Received	Not Recd
Bogusz, Stephen Jude Dr. Charles E McQueen		Division Of Medicine	3/08/95	9/07/97	Not Recd	Not Recd
Britton, Paul Dr. Frank C Tortella		Division Of Neuropsychiatry	7/15/94	3/28/97	Received	Received
Gandre, Helene Van Cu.. Dr. Charles H Hoke, Jr	(S)	Division Of Commun Diseases/Immunology	8/08/94	2/07/97	Received	Received
Gorbounov, Nikolai V Dr. Nabil M Elsayed		Division Of Medicine	6/06/94	6/05/97	Received	Not Recd
Kurtis, Jonathan David Dr. Patrick E Duffy		Division Of Commun Diseases/Immunology	7/01/96	6/06/97	Not Recd	Received
Lu, Xi-chun May Dr. Frank C Tortella		Division Of Neuropsychiatry	6/01/94	1/24/97	Received	Received
Luckhart, Shirley Dr. Ronald Rosenberg		Division Of Commun Diseases/Immunology	8/01/95	3/31/97	Not Recd	Received
Marek, Anne Maria Elis Dr. Ai J Lin		Division Of Experimental Therapeutics	2/12/96	2/28/97	Not Recd	Not Recd
Pakhomov, Andrew G Dr. Harry Zwick		Division Of Neuropsychiatry	1/26/94	1/25/97	Received	Received
Pardhasaradhi, Komandur Dr. Peter K Chiang	(S)	Division Of Biochemistry	5/12/94	10/23/96	Not Recd	Not Recd
Tsarev, Sergei Anatolyevich Dr. Bruce L Innis	(S)	Division Of Commun Diseases/Immunology	2/03/97	5/09/97	Not Recd	Not Recd
Zhang, Xiaoyan Dr. Marti Jett		Division Of Pathology	4/10/95	7/25/97	Received	Not Recd
Zhao, Bangti Dr. Joseph R Putnak	(S)	Division Of Commun Diseases/Immunology	1/10/94	4/09/97	Received	Received

14 Associates Listed

+ (S) indicates the associate was a Senior.

Name + Adviser	Division Citizenship	Starting Date	Ending Date
Asermely, Karen E.	Pathophysiology Division	5/22/95	11/21/97
Dr. Michael Adler	United States		
* Behonick, George Stanley	Pharmacology Division	8/04/97	8/03/98
Dr. Steven I Baskin	United States		
Keller, James Erich	Pharmacology Division	7/01/96	6/30/98
Dr. Margaret G Filbert	United States		
Morris, Jim	Pharmacology Division	9/11/95	9/10/98
Dr. Tsung-Ming A Shih	United States		

Total Associates Listed for Center: 4

* Indicates that the associate started tenure between 10/1/96 and 9/30/97.

+ (S) Associate is a Senior.

Name + Adviser	Division Citizenship	Starting Date	Ending Date
* Ahmed, Syed Ashraf (S)	Toxinology Division	8/18/97	8/17/98
Dr. Leonard A Smith	United States		
* Chen, Shin-Lin	Virology Division	10/01/97	9/30/98
Dr. John W Huggins	United States		
* Crise, Bruce Jeffrey	Division not specified	11/15/96	11/14/97
Dr. Michael D Parker	United States		
* Dailey, Frank (S)	Bacteriology Division	11/13/96	11/12/97
Dr. Arthur M Friedlander	United States		
Guttieri, Mary Charity	Virology Division	10/06/95	10/05/98
Dr. Connie S Schmaljohn	United States		
* Hatfill, Steven Jay (S)	Virology Division	9/18/97	9/17/98
Dr. John W Huggins	United States		
* Higgins, James A.	Division not specified	1/15/97	12/15/97
Dr. M S Ibrahim	United States		
Hooper, Jay William	Virology Division	7/05/95	7/04/98
Dr. Connie S Schmaljohn	United States		
Kamrud, Kurt Iver	Virology Division	8/05/96	8/04/98
Dr. Connie S Schmaljohn	United States		
Meyer, Barbara J	Virology Division	2/01/95	1/31/98
Dr. Connie S Schmaljohn	United States		
Pierson, Vicki Lynn D	Bacteriology Division	9/05/95	9/04/98
Dr. Patricia L Worsham	United States		
Pushko, Peter	Virology Division	5/20/94	11/19/97
Dr. Jonathan F Smith	Latvia		
* Reddy, Shanker P (S)	Bacteriology Division	3/03/97	3/02/98
Dr. Susan L Welkos	United States		
Saikh, Kamal Uddin (S)	Toxinology Division	4/03/95	4/02/98
Dr. Robert G Ulrich	India		
Wasieloski, Leonard P	Virology Division	4/24/95	4/23/98
Dr. Kevin Anderson	United States		
* Wilson, Julie Ann	Virology Division	3/24/97	3/23/98
Dr. Mary K Hart	United States		

Total Associates Listed for Center: 16

* Indicates that the associate started tenure between 10/1/96 and 9/30/97.

+ (S) Associate is a Senior.

Name + Adviser	Division Citizenship	Starting Date	Ending Date
* Moran, Daniel Sender Dr. Kent B Pandolf	Division not specified Israel	8/01/97	7/31/98
* Yeghiayan, Karine Sylva Dr. Harris R Lieberman	Division not specified United States	9/08/97	9/07/98

Total Associates Listed for Center: 2

* Indicates that the associate started tenure between 10/1/96 and 9/30/97.

+ (S) Associate is a Senior.

Associates On Tenure — October 1, 1997 — Attachment 2

AMRMC\Walter Reed Army Institute of Research — 10/17/97 Page 1 of 9

Name + Adviser	Division Citizenship	Starting Date	Ending Date
Abugo, Omoefe Oghenera. (S) Dr. Victor W Macdonald	Med Res Detachment-Blood Res Unit, MD Nigeria	4/06/95	10/05/97
Bhattacharjee, Apurba K (S) Dr. Jean M Karle	Division Of Experimental Therapeutics India	7/10/95	3/09/98
Chakrabarti, Arun Kumar (S) Dr. Prabhati Ray	Division Of Experimental Therapeutics India	5/30/96	1/29/98
* Cui, Ping Dr. Frank C Tortella	Division Of Neuropsychiatry People's Republic of China	7/28/97	7/27/98
* Das, Rina (S) Dr. Marti Jett	Division Of Pathology India	10/01/96	9/30/98
Ding, Xuan Zhou (S) Dr. Juliann G Kiang	Division Of Medicine People's Republic of China	10/03/94	1/02/98
Eze, Michael Okechukwu (S) Dr. David L Hoover	Division Of Commun Diseases/Immunology Nigeria	10/03/94	10/02/97
* Feaster, Shawn Ray Dr. Bhupendra P Doctor	Division Of Biochemistry United States	2/03/97	2/02/98
Fegeding, Konstantin V. Dr. Jeenan Tseng	Division Of Pathology Russia	10/16/95	10/15/98
* Fernandez-Prada, Carmen M Dr. David L Hoover	Division Of Commun Diseases/Immunology Peru	3/14/97	3/13/98
Fried, Michal Dr. Patrick E Duffy	Division not specified Israel	1/11/95	1/10/98
Gouvea, Vera S. (S) Dr. Bruce L Innis	Division Of Commun Diseases/Immunology United States	10/03/94	11/29/97
Guebre Xabier, Mimi (S) Dr. Urszula Krzych	Division Of Commun Diseases/Immunology Ethiopia	5/20/96	5/19/98
Li, Guo Dr. Harry Zwick	Med Res Detachment-Laser Res, TX People's Republic of China	9/16/96	9/15/98
Lin, Yu Dr. Joseph B Long	Division Of Neuropsychiatry People's Republic of China	7/18/94	1/17/98
Lumley, Lucille Ann Dr. James L Meyerhoff	Division Of Neuropsychiatry United States	1/03/96	1/02/98
Luo, Chunyuan Dr. Bhupendra P Doctor	Division Of Biochemistry People's Republic of China	3/12/96	3/11/98
* Ma, Da Dr. Raj K Gupta	Division Of Commun Diseases/Immunology People's Republic of China	1/29/97	1/28/98
Palmer, Dupeh Rachel O Dr. Urszula Krzych	Division Of Commun Diseases/Immunology England, U.K.	11/27/95	11/26/97

* Indicates that the associate started tenure between 10/1/96 and 9/30/97.
+ (S) Associate is a Senior.

Associates On Tenure October 1, 1997 **Attachment 2**

AMRMC\Walter Reed Army Institute of Research 10/17/97 [redacted]

Name + Adviser	Division Citizenship	Starting Date	Ending Date
Peel, Sheila Anne Dr. Rodger K Martin	Division Of Experimental Therapeutics United States	8/01/96	7/31/98
Ryu, Hyoik (S) Dr. Frederick J Cassels	Division Of Medicine Republic Of Korea	10/03/94	1/28/98
Santhanam, Kausalya Dr. Jayasree Nath	Division Of Medicine India	7/05/95	1/04/98
Yadava, Anjali Dr. Christian F Ockenhouse	Division Of Commun Diseases/Immunology India	1/02/96	1/01/98

Total Associates Listed for Center: 23

* Indicates that the associate started tenure between 10/1/96 and 9/30/97.

+ (S) Associate is a Senior.

Name/
Research Title

October 1996 Awardees

Fernandez-Prada, Carmen M
Generation of Isogenid Serum-Sensitive Brucella Melitensis Strains Through Transposon-Mediated Gene Disruption

Higgins, James A
Molecular Detection of Bacterial Pathogens Using a Novel 5' Nuclease Assay

Ma, Da
A New Approach to an Old Problem: High Tech Search for more Effective Mosquito Repellents

Reddy, Shanker P
Analysis of the Effects of the V Antigen and Plasminogen Activator of Yersinia Pestis on the Host Response ot Infectio

Yeghiayan, Karine S
Effects of Dietary L-Tryptophan and Stress on Neurochemistry and Performance

Name/
Research Title

February 1997 Awardees

Moran, Daniel S
Miniature Physiological Calculator (MPC) - A Tool for Predicting Soldier Performance

Wilson, Julie A
Cytotoxic T Lymphocyte Responses to Filovirus Proteins Expressed via Alphavirus Replicons

Name/
Research Title

June 1997 Awardees

Ahmed, Syed A
Structural and Functional Characterization of Botulinum Neurotoxin Domains

Behonick, George S
HPLC Detection of a Cyanide Metabolite: 4=O,omp Thiazolidine-2-Carboxylic Acid

Chen, Shin-Lin
Development of a Cell-Free Assay for the Inhibition of Ebola Virus Replication

Cui, Ping
Ischemic Brain Injury in Rats: Evaluation of Novel Neuroprotective Therapies

Hatfill, Steven J
Evaluation of Potential Therapeutic Interventions in Filovirus Infections

Phillips, James B, Jr
The Role of the Ubiquitin Proteasome Pathway in Secondary Injury of Neural Trauma

Xiang, Charlie C
Determination of Cellular Gene Expression During Filovirus Infection by cDNA Microarray Technology

October 1996

A- Accepted Award

FERNANDEZ-PRADA, CARMEN M — Ph.D. Date: 1996
Citizenship: Peru — George Washington University/DC
Adviser: Dr. David L Hoover — Actual Starting Date: 3/14/97
Research Field: Microbiology — Termination Date: 3/13/98
Research Title: Generation of Isogenid Serum-Sensitive Brucella Melitensis Strains Through Transposon-Mediated Gene Disruption

HIGGINS, JAMES A — Ph.D. Date: 1992
Citizenship: United States — Johns Hopkins University/MD
Adviser: Dr. M S Ibrahim — Actual Starting Date: 1/15/97
Research Field: Infectious Diseases — Termination Date: 12/15/97
Research Title: Molecular Detection of Bacterial Pathogens Using a Novel 5' Nuclease Assay

MA, DA — Ph.D. Date: 1996
Citizenship: People's Republic Of China — Howard University/DC
Adviser: Dr. Raj K Gupta — Actual Starting Date: 1/29/97
Research Field: Organic Chemistry — Termination Date: 1/28/98
Research Title: A New Approach to an Old Problem: High Tech Search for more Effective Mosquito Repellents

REDDY, SHANKER P — Ph.D. Date: 1987
Citizenship: United States — University of Florida
Adviser: Dr. Susan L Welkos — Actual Starting Date: 3/03/97
Research Field: Infectious Diseases — Termination Date: 3/02/98
Research Title: Analysis of the Effects of the V Antigen and Plasminogen Activator of Yersinia Pestis on the Host Response ot Infection

YEGHIAYAN, KARINE S — Ph.D. Date: 1995
Citizenship: United States — Northeastern University/MA
Adviser: Dr. Harris R Lieberman — Actual Starting Date: 9/08/97
Research Field: Neurosciences — Termination Date: 9/07/98
Research Title: Effects of Dietary L-Tryptophan and Stress on Neurochemistry and Performance

February 1997

A- Accepted Award

MORAN, DANIEL S — Ph.D. Date: 1993
Citizenship: Israel — Tel Aviv University/Israel
Adviser: Dr. Kent B Pandolf — Actual Starting Date: 8/01/97
Research Field: Environmental Medicine — Termination Date: 7/31/98
Research Title: Miniature Physiological Calculator (MPC) - A Tool for Predicting Soldier Performance

WILSON, JULIE A — Ph.D. Date: 1996
Citizenship: United States — U of Florida College of Medicine
Adviser: Dr. Mary K Hart — Actual Starting Date: 3/24/97
Research Field: Viral Immunology — Termination Date: 3/23/98
Research Title: Cytotoxic T Lymphocyte Responses to Filovirus Proteins Expressed via Alphavirus Replicons

8- Declined

BLACK, CHARLES A, JR — Ph.D. Date: 1997
Citizenship: United States — Newcastle, U
Adviser: Dr. Mark T Dertzbaugh
Research Field: Immunology
Research Title: Mucosal DNA Vaccination Against Botulinum Neurotoxin

June 1997

A- Accepted Award

AHMED, SYED A — Ph.D. Date: 1983
Citizenship: United States — Kyoto University/Japan
Adviser: Dr. Leonard A Smith — Actual Starting Date: 8/18/97
Research Field: Life Science — Termination Date: 8/17/98
Research Title: Structural and Functional Characterization of Botulinum Neurotoxin Domains

BEHONICK, GEORGE S — Ph.D. Date: 1997
Citizenship: United States — St. John's Univ-Staten Island/NY
Adviser: Dr. Steven I Baskin — Actual Starting Date: 8/04/97
Research Field: Pharmacology Toxicology — Termination Date: 8/03/98
Research Title: HPLC Detection of a Cyanide Metabolite: 4=O,omp Thiazolidine-2-Carboxylic Acid

CHEN, SHIN-LIN — Ph.D. Date: 1992
Citizenship: United States — University of Minnesota-Twin Cit
Adviser: Dr. John W Huggins — Actual Starting Date: 10/01/97
Research Field: Molecular Virology — Termination Date: 9/30/98
Research Title: Development of a Cell-Free Assay for the Inhibition of Ebola Virus Replication

CUI, PING — Ph.D. Date: 1990
Citizenship: People's Republic Of China — Tianjin Medical College/China
Adviser: Dr. Frank C Tortella — Actual Starting Date: 7/28/97
Research Field: Neurosciences — Termination Date: 7/27/98
Research Title: Ischemic Brain Injury in Rats: Evaluation of Novel Neuroprotective Therapies

HATFILL, STEVEN J — Ph.D. Date: 1994
Citizenship: United States — Rhodes U
Adviser: Dr. John W Huggins — Actual Starting Date: 9/18/97
Research Field: Experimental Medicine — Termination Date: 9/17/98
Research Title: Evaluation of Potential Therapeutic Interventions in Filovirus Infections

PHILLIPS, JAMES B, JR — Ph.D. Date: 1997
Citizenship: United States — Uniformed Services U Hlth Sci-Unk
Adviser: Dr. Frank C Tortella — Actual Starting Date: 10/06/97
Research Field: Neuropharmacology — Termination Date: 10/05/98
Research Title: The Role of the Ubiquitin Proteasome Pathway in Secondary Injury of Neural Trauma

XIANG, CHARLIE C — Ph.D. Date: 1994
Citizenship: Canada — Waterloo, U-Ont
Adviser: Dr. Kevin Anderson — Expected Starting Date: 5/01/98
Research Field: Virology — Termination Date: 4/30/99
Research Title: Determination of Cellular Gene Expression During Filovirus Infection by cDNA Microarray Technology

On Tenure Report by Quarter and Center | For the year starting **October 1, 1996** | **Attachment 5** 9/11/97 [redacted]

U.S. Army Medical Research and Materiel Command

Center	Number of Associates on tenure as of					
	10/1/95	10/1/96	1/1/97	4/1/97	7/1/97	10/1/97
Medical Res Inst of Chemical Defense	3	4	4	4	4	4
Medical Research Institute for Infectious Diseases	20	15	15	16	13	15
Research Institute of Medical Sciences	1	1	1	0	0	0
U.S. Army Institute of Surgical Research	1	0	0	0	0	0
U.S. Army Research Institute of Environmental Medicin	2	3	2	2	2	2
Walter Reed Army Institute of Research	32	32	30	28	24	22
	59	55	52	50	43	43

[r_tenure_by_quarter]

FINAL REPORT

(1) DATE
21 March 1997

(2) NAME
Paul Britton (97.15.50.11)

(3) NAME OF LAB OR CENTRE AND LOCATION
Walter Reed Army Institute of Research, Building 40,
Washington, DC 20307-5100

(4) DATES OF TENURE
14 July 1994 - 28 March 1997

(5) TITLE OF RESEARCH PROPOSAL
Neurochemical alterations at sites of ischemic insult in rats: effects and evaluation of putative neuroprotective agents.

(6) NAME OF RESEARCH ADVISOR
Dr. Frank C. Tortella

(7) ARE YOU ON LEAVE FROM A PROFESSIONAL POST?
No

(8) PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE
N/A

(9) PROFESSIONAL TRAVEL DURING TENURE
24th Annual meeting of the Society for Neuroscience. Miami, FL. Nov. 13-18, 1994.
British Pharmacological Society Meeting. Glasgow, Scotland. Sep. 6-8, 1995.
25th Annual meeting of the Society for Neuroscience. San Diego, CA. Nov. 11-16, 1996.
Experimental Biology meeting. Washington, DC. Apr. 14-17, 1996.
26th Annual meeting of the Society for Neuroscience. Washington, DC. Nov. 16-21, 1996.
American Society for Pharmacology and Experimental Therapeutics meeting. San Diego, CA. Mar. 7-11, 1997.

(10) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES
N/A

(11) SUMMARY OF RESEARCH DURING TENURE
The aims of the original research proposal have been vigorously pursued during the 32

month period of tenure at WRAIR. With respect to the medical interests of the US Army in CNS injury and Combat Casualty Care, a "non-invasive" rat model of focal ischemic brain injury has been developed. Using this paradigm we have demonstrated not only the varying infarct volumes resulting from permanent or transient (with reperfusion) focal ischemia, but also the differential neuroprotective effects of dextromethorphan (DM) in these methodologies. Use of a Loats Image Analysis System has enabled the computer aided evaluation of brain infarct volumes following transient or permanent ischemia. In particular, three dimensional imaging strategies have been used to potentially locate and evaluate the effect of DM on ischemic core and penumbral areas. In combination with this, an in vivo microdialysis procedure which allows multisite monitoring of changes in excitatory amino acids in the ischemic core and perifocal penumbral tissue, both in the absence and presence of neuroprotective agents has been develop ed. Using a mouse model of focal brain injury we have evaluated the effect of heat-shock protein in brain injury following focal cerebral ischemia and examined the potential neuroprotective effect of the angiogenesis drug thalidomide. Using the rat model of transient MCAO we are currently examining the potential neuroprotective actions of 2 novel drugs, namely SNC 100 and AHN 649 in an attempt to establish i.v. neuroprotective dose-response profiles for these agents.

(12) RESEARCH IN PROGRESS
N/A

(13) PRESENTATION AT SCIENTIFIC MEETINGS OR CONFERENCES

Tortella, F., Bevan, K., Bowery, N., Britton, P., Lu, M., Newman, A., and Calderone, S. (1995). SNC 100: A novel potent neuroprotective analog of carbetapentane. (Abstract for the 3rd International Neurotrauma Symposium, July 22-27, 1995).

Britton, P., Lu, X.-C.M., Lloyd, D., Gribben, S., Loats., and Tortella, F. (1995). Neuroprotective effect of dextromethorphan following transient, but not permanent, focal cerebral ischemia in the rat. Soc. Neurosci. Abstr. Vol 21, Part 2, 993.

Lu, X.-C.M., Britton, P., Clapp, L., Johnston, J., and Tortella, F. (1995). EEG analysis of focal ischemic injury in the rat. Soc. Neurosci. Abstr. Vol 21, Part 1, 224.

Ray, J.P., Britton, P., Lu, X.-C.M., Tortella, F.C., and Dave, J.R. (1995). In situ hybridization studies of ß-actin gene expression in rat brain tissue following ischemic injury and reperfusion. Soc. Neurosci. Abstr. Vol 21, Part 2, 993.

Britton, P., Dave, J.R., Lu, X-C.M., Laskosky, M.S., Mestril, R., Dillman, W.H. and Tortella, F.C. (1996). Effect of focal cerebral ischemia in a transgenic mouse strain overexpressing the rat inducible 70-KD heat shock protein (rHSP70i). The FASEB Journal, 10, A1511.

Dave, J.R., Tortella, F.C., Britton, P., Rusakov, S.A., Doctor, B.P. and Ved, H.S. (1996). Heat-hock mediated protection against glutamate toxicity in neuronal cultures derived

from different regions of rat brain. Medical Defense Bioscience Review, Baltimore, MD, 12-16 May.

Britton, P., Dave, J.R., Lu, X.-C.M., Ray, J.P., Mestril, R., Dillman, W.H., Ved, H.S. and Tortella, F.C. (1996). Heat-shock protein (HSP70) involvement in glutamate neurotoxicity and focal cerebral ischemia: a transgenic mouse strain overexpressing the rat inducible HSP70. (To be presented at the 26th Annual Meeting of the Society for Neuroscience, Washington, DC, Nov 16-21.)

Britton, P. and Tortella, F.C. (1997). A novel method of in vivo microdialysis following transient focal cerebral ischemia: continuous sampling of extracellular fluid (ECF) from multiple sites within the ischemic territory in unanaesthetized rats. (Presented at the ASPET meeting, San Diego, CA, Mar 7-11)

Tortella, F.C., Lu, X.-C.M., Newman, A.H. and Britton, P. (1997). Post-treatment with the novel dextromethorphan (DM) analog AHN649, dose-dependently reduces infarct size following temporary focal ischemia in rats. (Presented at the ASPET meeting, San Diego, CA, Mar 7-11)

(14) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH

Tortella, F., Bevan, K., Bowery, N., Britton, P., Lu, M., Newman, A., and Calderone, S. (1995). SNC 100: A novel potent neuroprotective analog of carbetapentane. (Abstract for the 3rd International Neurotrauma Symposium, July 22-27, 1995).

Britton, P., Lu, X.-C.M., Lloyd, D., Gribben, S., Loats., and Tortella, F. (1995). Neuroprotective effect of dextromethorphan following transient, but not permanent, focal cerebral ischemia in the rat. Soc. Neurosci. Abstr. Vol 21, Part 2, 993.

Lu, X.-C.M., Britton, P., Clapp, L., Johnston, J., and Tortella, F. (1995). EEG analysis of focal ischemic injury in the rat. Soc. Neurosci. Abstr. Vol 21, Part 1, 224.

Ray, J.P., Britton, P., Lu, X.-C.M., Tortella, F.C., and Dave, J.R. (1995). In situ hybridization studies of ß-actin gene expression in rat brain tissue following ischemic injury and reperfusion. Soc. Neurosci. Abstr. Vol 21, Part 2, 993.

Britton, P., Dave, J.R., Lu, X-C.M., Laskosky, M.S., Mestril, R., Dillman, W.H. and Tortella, F.C. (1996). Effect of focal cerebral ischemia in a transgenic mouse strain overexpressing the rat inducible 70-KD heat shock protein (rHSP70i). The FASEB Journal, 10, A1511.

Dave, J.R., Tortella, F.C., Britton, P., Rusakov, S.A., Doctor, B.P. and Ved, H.S. (1996). Heat-hock mediated protection against glutamate toxicity in neuronal cultures derived from different regions of rat brain. Medical Defense Bioscience Review, Baltimore,

MD, 12-16 May.

Britton, P., Dave, J.R., Lu, X.-C.M., Ray, J.P., Mestril, R., Dillman, W.H., Ved, H.S. and Tortella, F.C. (1996). Heat-shock protein (HSP70) involvement in glutamate neurotoxicity and focal cerebral ischemia: a transgenic mouse strain overexpressing the rat inducible HSP70. (To be presented at the 26th Annual Meeting of the Society for Neuroscience, Washington, DC, Nov 16-21.)

Britton, P. and Tortella, F.C. (1997). A novel method of in vivo microdialysis following transient focal cerebral ischemia: continuous sampling of extracellular fluid (ECF) from multiple sites within the ischemic territory in unanaesthetized rats. (To be presented at the ASPET meeting, San Diego, CA, Mar 7-11)

Tortella, F.C., Lu, X.-C.M., Newman, A.H. and Britton, P. (1997). Post-treatment with the novel dextromethorphan (DM) analog AHN649, dose-dependently reduces infarct size following temporary focal ischemia in rats. (To be presented at the ASPET meeting, San Diego, CA, Mar 7-11)

Britton, P. and Tortella, F.C. Differential mouse strain susceptibility to focal cerebral ischemia: effect of MK 801 on resulting brain injury. (Accepted for publication in *Pharmacology Communications*, Sep, 1996).

Britton, P., Lu, X.-C.M., Laskosky, M.S., and Tortella, F.C.(1997) Dextromethorphan protects against cerebral injury following transient, but not permanent, focal ischemia in rats. *Life Sciences*. 60 (20), 1729-1740.

Tortella, F.C., Lu, X-C.M., Britton, P., Laskosky, M., Rose, J., Robles, L., DeCoster, M. and Newman, A.H. AHN649, a novel analog of dextromethorphan, is neuroprotective and improves recovery following global ischemia in rats. (Accepted for publication in *Pharmacology Communications*, Nov, 1996).

(15) PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH
N/A

(16) FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS
Research Associate
The Advisory Board Company
The Watergate
New Hampshire Avenue, NW
Washington, DC 20037

(17) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS
I found the Associateship program to be efficient and effective in all their functions and roles to provide support for me as an NRC associate. I would highly recommend the

Associateship program to any post-doctoral research fellow.

05.05.97

PII Redacted

Dr. Nikolai V. Gorbounov [redacted]
Senior Researcher,
On leave from IM Sechenov Institute of
the Evolutionary Physiology and Biochemistry,
The Russian Academy of Sciences, S. Petersburg,
Russia

Department Respiratory Research,
Division of Medicine,
Walter Reed Army Institute of Research
Washington, D.C.

Date of Tenure: June 5, 1994 - June 5, 1997

Title of Research Proposal:
The oxygen intermediates in the molecular mechanism of oxidative damage to the lung produced upon exposure to the air blast overpressure.

Research Adviser: Dr. N.M. Elsayed

Professional Society Offices Held During Tenure:
(1) The Oxygen Society, (2) Society for Experimental Biology and Medicine

Summary of Research During Tenure:

The air blast overpressure (BOP) induced damage to the lung is characterized by destroy of the alveolar/capillary barrier leading to early pulmonary hemorrhage, hematoma, alveolar penetration of erythrocytes, and oxidative stress. The BOP-induced oxidative stress in the lung is due to extravascular release of oxyhemoglobin inducing free radical peroxidation. This effect is accompanied by depletion of antioxidants in the lung tissue and blood, and leads to the formation of the mediators of oxidative stress and inflammatory response, namely, oxygen-derived reactive species (ODRS), lipid peroxidation products (LPO), and niric oxide. The secondary oxidative damage to circulatory system produced by ODRS and LPO can be alleviated by nitric oxide.

Rsearch in Progress:

The experimental part of the research has been completed. The experimental results have been published, or prepared for publication (see the list of publications).

N. Gorbounov

Professional Travel During Tenure:

National Meetings and Conferences:

An Annual Meeting of Professional Research Scientists "Experimental Biology 95"' (Atlanta, Georgia, April 9-13, 1995). Presenter.

VII International Congress of Toxicology (Seattle, Jul. 2-6, 1995). Presenter.

Annual Meeting of American Society of Toxicology 1996 (Anaheim, March 10-14 1996).
Presenter.

An Annual Meeting of Professional Research Scientists "Experimental Biology 96"' (Bethesda, Maryland, April 14-17, 1996).
Presenter.

4th Biennial International Symposium on "Alternatives in the Assessment of Toxicity: Issues, Progress and Opportunities." (Research , Development and Engineering Center, Edgewood, Maryland, June 12-14, 1996).
Presenter.

An Annual Meeting of the Oxygen Society, "Oxygen '96" (Miami Beach, Florida, November 21-25, 1996).
Presenter.

International Conference:

IUBMB Conference "'The Life & Death of the Cell" Edinburgh International Conference Center, July 14-17, 1996.
Presenter.

Seminars Delivered at Universities and Institutes:

" The role of NO in hemoglobin-generated oxidative stress." Workshop on "The Molecular Mechanism(s) of Blast Overpressure-Induced Injury." October 26, 1994, Walter Reed Army Institute of Research.

"Antioxidant function of nitric oxide against hemoglobin-catalyzed oxidation: Electron Spin Resonance Studies." Symposium on The New Approches to Mechanism-Based Therapies Against Blast Overpressure-Induced Injury." February 9, 1996, Walter Reed Army Institute of Research.

"Hemoglobin in the mechanism of pulmonary oxidative damage produced upon exposure to air blast." Invited lecture. January 16, 1996. Department of the Environmental and Occupational Health, the University of Pittsburgh, Pittsburgh, Pennsylvania.

Publications and Papers Resulting from NRC Associateship Research:

(a) Publications in peer-reviewed journals:

Gorbunov, NV, Osipov, AN, Day, BW, Zayas-Rivera, B, Kagan, VE, Elsayed, NM. Reduction of ferrylmuoglobin and ferrylhemoglobin by nitric oxide: a protective mechanism against ferryl hemoprotein-induced oxidations. Biochemistry, 34(20), 6689-6699,1995.

Osipov, AN, Gorbunov, NV, Day, BW, Elsayed, NM, Kagan, VE. Electron Spin Resonance and Mass Spectral Analysis of the Interactions of Ferrylhemoglobin and Ferrylmyoglobin with Nitric Oxide. Meth. Enzimol. 268(18), 193-203, 1996.

Maulik, N., Engelman, D.Y., Watanable, M., Engelman, R.M., Rousou, J.A., Flack, J.A., Deaton, D.W., Gorbunov, N., Elsayed, N.M., Kagan, V.E., Das, D.K. Nitric oxide /carbon monoxide: molecular switch for myocardial preservation during ischemia. Circulation. Supplement II. 94(9), II-398 - II-406, 1996.

Kagan, VE, Day, BW, Elsayed, NM, Gorbunov, NV.
Dynamics of Nitrosylated Haemoglobin in Blood.
Nature *(London)*. 383: 30-31, 1996

Gorbunov, NV, Osipov, AN, Sweetland, MA, Day, BW, Elsayed, NM, Kagan, VE. NO Redox Paradox: Direct Oxidation of α-Tocopherol and α-Tocopherol-Mediated Oxidation of Ascorbate. Biochem. Biophys. Res. Commun. 219, 835-841, 1996.

Gorbunov, NV., Yalowich, JC., Gaddam, A., Thampatty, P., Ritov, VB., Kisin, ER., Elsayed, NM., Kagan, VE. Nitric oxide prevents oxidative damage produced by tert-butylhydroperoxide in erythroleukemia cells via nitrosylation of heme and non-heme iron: electron paramagnetic resonance evidence.J. Biological Chemistry. 272(19): 12328-12341, 1997.

Gorbunov, N.V., Elsayed, N.M., Kisin, E.R, Kozlov, A.V., Kagan, V.E. Air blast overpressure induces oxidative stress in the rat lung: interplay between hemoglobin, antioxidants and lipid peroxidation. Am. J. Physiol. 272(Lung Cell. Mol. Physiol. 16), L320-L334, 1997.

(c) Manuscripts in preparation, manuscripts submitted:

Kagan, V., Gorbunov, N. EPR measurements of nitric oxide-induced chromanoxyl radicals of vitamin E: Interactions with vitamin C. In: Methods in Molecular Biology. The Humana Press INC. Ed. Armstrong, D.

Yalowich, JC., Gorbunov, NV., Kagan, VE. Both post-transcriptionally decreased levels of heme- and non-heme iron and direct reaction of NO at catalytic iron-sites protect iNOS-transfected human erythroleukemia cells against tert-butyl hydroeroxide.

Gorbunov, NV., Tyurina, Yu., Salama, G., Argiros, G., Day, B., Elsayed, NM., Kagan VE. Nitric oxide prevents *tert*-butyl hydroperoxide induced formation of free radicals and oxidative damage to cardiac myocytes.

Presentations at Scientific Meetings and Conferences:

Gorbounov, NV, Osipov, AN, Day, BW, Kagan, VE, Elsayed, NM. Nitric oxide protects from oxidative stress by reducing ferryl myoglobin and ferryl hemoglobin radicals. FASEB J.(Experimental Biology, Abstracts (II)), 9(4), 5175,1995. An Annual Meeting of Professional Research Scientists "Experimental Biology 95"' (Atlanta, Georgia, April 9-13, 1995).

Gorbunov, NV, Osipov, AN, Day, BW, Zayas-Rivera, B, Kagan, VE, Elsayed, NM. Antioxidant function of nitric oxide against oxoferryl - induced oxidations in the presence of organic hydroperoxides. In: Abstracts of the VII International Congress of Toxicology (Seatle, July 2-6, 1995), 7(1), 48-PF-2,1995.

Elsayed, NM, Smith, D, Ebel, D, Gorbunov, NV, Topper, MJ, Kagan, VE, Mayorga, MA. Pulmonary alterations after brief, nose-only exposure of rats to high-level nitrogen dioxide. In: Abstracts of the VII International Congress of Toxicology (Seatle, July. 2-6, 1995), 7(1), 19-P-6,1995.

Elsayed, NM., Kagan, VE., Gorbunov, NV., Kozlov, AV., Ratov, VB., Gaddam, A., Mawhinney, BJ., Yalowich, J.C."The antioxidant paradox: prooxidant function of water soluble antioxidants, and antioxidant functions for nitric oxide against blast overpressure-induced oxidative stress." Invited abstract at 9th Gordon Research Conference "Oxygen Radicals in Biology". 11-16 February. Ventura, California. U.S.A.

Gorbunov, NV., Kagan, VE., Elsayed, NM., Kozlov, AV., Ritov, VB., Gaddam, A., Mawhinney, BJ., Yalowich, JC. A novel antioxidant function of nitric oxide against oxidative damage induced by hemoglobin. The Toxicologist. 30, 1(2), 312, 1996. Annual Meeting of American Society of Toxicology 1996 (Anaheim, March 10-14 1996).

Gorbunov, NV., Elsayed, NM., Kisin, ER., Ritov, VB., Gaddam, A., Allan WP., Yalowich, JC., Kagan, VE. Nitrosylation of hemoglobin prevents alkylhydroperoxide- induced oxidative stress in erythroleukemia cells. FASEB J. (Abstracts) 10: 1160, 1996. An Annual Meeting of Professional Research Scientists "Experimental Biology 96"' (Bethesda, Maryland, April 14-17, 1996).

Gorbunov, NV., Elsayed, NE., Kisin, ER., Gaddam, A., Yalowich, JC., Kagan, VE. Erythropoietic K/VP.5 cells as a model for testing antioxidant effectiveness of nitric oxide against hydroperoxide cytotoxicity. 4th Biennial International Symposium on "Alternatives in the Assessment of Toxicity: Issues, Progress and Opportunities." (Research , Development and Engineering Center, Edgewood, Maryland, June 12-14, 1996).

Gorbunov, NV., Yalowich, JC., Gaddam, A., Elsayed, NM., Kagan, VE. "Formation of Iron-Nitrosyl Complexes Protects K/VP.5 Cells Against tert-Butylhydroperoxide-Induced Oxidative Stress." IUBMB Conference ""The Life & Death of the Cell" Edinburgh International Conference Center, July 14-17, 1996.

Kagan, VE., Gorbunov, NV., Gaddam, A., Kisin, ER., Elsayed, NM., Yalowich, JC. " A Novel Antioxidant Mechanism of Nitric Oxide in Human Erythroleukemia Cells. " Invited presentation at "VIII Biennial Meeting International Society for Free Radical Research." 1-5 October, 1996. Barcelona. Spain.

Gorbunov, N. V. , Menshikova, E.V., Salama, G. , Argyros, G.J., Elsayed, N.M. , Claycamp, H.G., Kagan, V.E. Nitric oxide prevents free radical damage to cardiac sarcoplasmic reticulum produced by tert-butylhydroperoxide and myoglobin. In: Abstracts of An Annual Meeting of the Oxygen Society, "Oxygen '96" (Miami Beach, Florida, November 21-25, 1996).

Gorbunov, NV, Menshikova, E.V., Salama, G., Argiros, N.M., Elsayed, N.M., Claycamp, H.G., Kagan, VE. Nitric oxide prevents tert-butyl hydroperoxide induced formation of peroxyl radicals in cardiomyocytes: role of endogenous iron. The Toxicologist. 36, 1(2), 312, 1997. 36th Annual Meeting of The Society of Toxicology (Cincinnati, Ohio, March 9-13, 1997)

Elsayed, NM., Gorbounov, NV., Viduya, JC., Grant, PC., Morris, JR., Armstrong, KL., and Kagan, VE. Pulmonary injury from repeated exposure to blast overpressure. In: Abstracts of 1997 International Conference (American Lung Association), (Am. J. Respiratory and Critical Care Medicine, 155(4), A580, 1997) May 16-21, 1997, San Francisco, CA)

05.05.97

PII Redacted

Dr. Nikolai V. Gorbounov [redacted]
Senior Researcher,
On leave from IM Sechenov Institute of
the Evolutionary Physiology and Biochemistry,
The Russian Academy of Sciences, S. Petersburg,
Russia

Department Respiratory Research,
Division of Medicine,
Walter Reed Army Institute of Research
Washington, D.C.

Date of Tenure: June 5, 1994 - June 5, 1997

The NRC Program:
"Oxidative Stress and Molecular Mechanisms of Injury and Repair"
#97.15.40.10

Title of Research Proposal:
The oxygen intermediates in the molecular mechanism of oxidative damage to the lung produced upon exposure to the air blast overpressure.

Research Adviser: Dr. N.M. Elsayed

Professional Society Offices Held During Tenure:
(1) The Oxygen Society, (2) Society for Experimental Biology and Medicine

Summary of Research During Tenure:

The air blast overpressure (BOP) induced damage to the lung is characterized by destroy of the alveolar/capillary barrier leading to early pulmonary hemorrhage, hematoma, alveolar penetration of erythrocytes, and oxidative stress. The BOP-induced oxidative stress in the lung is due to extravascular release of oxyhemoglobin inducing free radical peroxidation. This effect is accompanied by depletion of antioxidants in the lung tissue and blood, and leads to the formation of the mediators of oxidative stress and inflammatory response, namely, oxygen-derived reactive species (ODRS), lipid peroxidation products (LPO), and nitric oxide. The secondary oxidative damage to circulatory system produced by ODRS and LPO can be alleviated by nitric oxide.

Rsearch in Progress:

The experimental part of the research has been completed. The experimental results have been published, or prepared for publication (see the list of publications).

N. Gorbounov

Patents Resulting from NRC Associateshop Research:

No patents resulting from associateship research.


N. Gorbounov

Final Report

Date:	January 22, 1997
Name:	Xi-Chun May Lu
Laboratory:	Walter Reed Army Institute of Research
Tenure dates:	July 2, 1996 - January 24, 1997
Title of Proposal:	Ischemic Brain Injury in Rats: Evaluation of Novel Neuroprotective Therapies
Name of Adviser:	Dr. Frank C. Tortella
Professional Society:	Member of Society for Neuroscience
Professional Travel:	to Annual meetings of Society for Neuroscience (1994, 1995, 1996)
Siminars:	Guilford Pharmaceutical, Inc., Baltimore, December 12, 1996

Summary of Research:

1. Established a rodent model of focal cerebral ischemic injury which is produced by intraluminal filament occlusion of middle cerebral artery (MCAO) and evaluated by-topographic EEG spectral analysis. During the first 24 hr MCAO, severe ipsilateral voltage depression and wave-form slowing developed, but the pattern of abnormality was clearly spatially differentiated. 2. Using this model to evaluated the neuroprotective effect of Dextramethorphan, which showed significant reduction of infarct volume in transient, but not in permanent, MCAO. 3. Demonstrated neuroprotective effect of *c-fos* antisense oligonucleotide against NMDA-induced excitotoxicity in an *in vivo* model.

Research in Progress:

Evaluating neuroprotective effect of DM's analogs and other anticonvulsant compounds in the MCAO model using histopathological measurements and EEG topographic mapping .

Presentations:

Lu, X.-C. M., Dave, J. R., Laskosky, M. S., Ved, H. S. and Tortella, F. C. Neuroprotective role of *c-fos* antisense oligonucleotide against glutamate/NMDA induce neurotoxicity in the rat hippocampus. Poster presentation at 26th Annual Meeting of Society for Neuroscience. (Abstr, 22(1): 799, 1996).

Britton, P., Dave, J. R., Lu, X.-C. M., Ray, J. P., Mestril, R., Dillman, W. H. Ved, H. S. and Tortella, F. C. Heat-shock protein (HSP70) involvement in glutamate neurotoxicity and focal cerebral ischemia: a transgenic mouse strain overexpressing the rat inducible HSP70. Poster presentation at 26th Annual Meeting of Society for Neuroscience. (Abstr, 22(3): 1673, 1996).

Britton, P., Dave, J. R., Lu, X.-C. M., Laskosky, M. S., Mestril, R., Dillman, W. H. and Tortella, F. C. Effect of focal cerebral ischemia in a transgenic mouse strain overexpressing the rat inducible 70-KD heat shock protein (rHSP70i). Fed. Proc. 10: A263, 1996.

Lu, X.-C. M., Britton, P., Clapp, L., Jonstone, J. and Tortella, F. C. EEG analysis of focal ischemic injury in the rat. Poster presentation at 25th Annual Meeting of Society for Neuroscience. (Abstr., 21(1): 224, 1995).

Britton, P., Lu., X.-C. M., Lloyd, D., Gribben, S., Loats, H. and Tortella, F. C. Neuroprotective effect of dextromethorphan following transient, but not permanent, focal cerebra ischemia in the rat. Poster presentation at 25th Annual Meeting of Society for Neuroscience. (Abstr., 21(2): 995, 1995).

Ray, J. P., Lu, X.-C. M., Tortella, F. C. and Dave, J. R. *In Situ* hybridization studies of β-actin gene expression in rat brain tissue following ischemic injury and reperfusion. Poster presentation at 25th Annual Meeting of Society for Neuroscience. (Abstr., 21(2): 993, 1995).

Tortella, F. C., Bevan, K., Bowery, N., Britton, P., Lu, X.-C. M., Newman, A. H. and Calderon, S. SNC 100: A novel potent neuroprotective analog of carbetapentate. 3rd International Neurotrauma Symposium, Toronto, Canada, 22-27 July, 1995.

Publications:

Lu, X.-C. M., Britton, P., and Tortella, F. C. Topographic EEG spectral analysis of focal cerebral ischemic injury in the rat MCAO model. (manuscript in preparation for publication).

Lu, X.-C. M., Tortella, F. C., Ved, H. S. and Dave J. R. Neuroprotective Role of *c-fos* antisense oligonucleotide: *in vivo* and *in vitro* studies, Submitted for publication, 1997.

Tortella, F. C., Lu, X.-C. M., Britton, P., Laskosky, M. , Rose, J., Robles, L., DeCoster, M., Newman, A. H. AHN649, a novel analog of dextromethorphan, is neuroprotective and improves recovery following global forebrain ischemia in rats. Submitted for publication, 1996.

Britton, P., Lu, X.-C. M., Laskosky, M. S., Tortella, F. C. Dextromethorphan protects against cerebral injury following transient, but not permanent, focal ischemia in rats. Submitted for publication, 1996.

Future position: Scientist at Guilford Pharmaceutical Inc.
Department of Research
6611 Tributary Street
Baltimore, MD 21224

Comments:

I greatly appreciate the opportunity of doing research at Walter Reed Army Institute of Research as a regular NRC research associate. The program provides sufficient supervision from the laboratory and the freedom for the individual to do research of interests of both parties. Personally I have acquired tremendous working experiences in research on ischemia/stroke brain injury, which has laid the foundation for my future career in this field. I also believe that my performance at WRAIR is beneficial to the institute. I am very thankful for the program administrators on any assistance I needed during the past years.

9293630

FINAL REPORT

1. Date:

1-10-1997

2. Name:

Andrei G Pakhomov

3. Name of Laboratory or Center and Location:

Microwave Bioeffects Branch
US Army Medical Research Detachment
of the Walter Reed Army Inst. of Research
8308 Hawks Road, Bldg. 1168
Brooks AFB, TX 78235

4. Dates of Tenure

January 25, 1994 - January 25, 1997

5. Title of the Research Proposal:

"Mechanisms of extremely-high frequency electromagnetic radiation effect on nervous functions"

6. Name of Research Adviser

Col. C. B. G. Campbell (till July 1996)

Dr. Harry Zwick (after July 1996)

7. Are You on Leave from a Professional Post?

Yes. ***Leading Scientist*** of the Department of Pharmacology, Medical Radiology Research Center, Russian Academy of Medical Sciences, Obninsk, Kaluga region, 249020 Russia

8. Professional Society Offices Held During Tenure:

Bioelectromagnetics Society (**BEMS**)

European Bioelectromagnetic Association (**EBEA**)

Physiological Society of Russia (**PSR**)

9. Professional Travel During Tenure:

1. Domestic

August 1995 2nd Annual Michaelson Research Conf., Kalispell, MO.

June 1995 — 17th Annual Meeting of Bioelectromagnetics Society, Boston, MA.

April 1994 — Site visit to the Center for Biomed. Physics, Temple Univ. School of Medicine, Philadelphia, PA.

2. Foreign

June 1996 — 18th Annual Meeting of Bioelectromagnetics Society, Victoria, BC, Canada

March 1996 — 3rd EBEA Congress, Nancy, France

August 1994 — 5th World Congress on Med. Physics and Biomed. Engineering, Rio de Janeiro, Brazil.

June 1994 — Site visit with Col. E. C. Elson (WRAIR) and Dr. M. Murphy (Armstrong Lab) to Russian institutes.

10. Seminars or Lectures Delivered at Universities and/or Institutes:

"Search for Microwave-Sensitive Structures and Functions in the Nervous System" and "Microwave Influence on the Isolated Heart Function." - both presented at the Temple Univ. School of Medicine, Philadelphia, PA. (1994)

11. Summary of Research During Tenure

Experiments on isolated sciatic nerve established a nonthermal, frequency-specific effect of millimeter wave (MMW) radiation. This effect was an attenuation of the decrease of compound action potentials under a high-rate electrical stimulation of the nerve. Dependencies of the effect upon frequency and intensity of the radiation have been analyzed. The nonthermal effect has recently been confirmed in independent experiments on the isolated hemisected frog spinal cord. The results of the study contribute to current knowledge of electromagnetic field bioeffects, and are important for hygienic standardization of MMW exposure.

12. Research in Progress:

Analysis of neurophysiological mechanisms of MMW effect on neurone circuitry function.

13. Presentations at Scientific Meetings and Conferences:

1. **Pakhomov, A.G.** Millimeter Wave Medicine in Russia: A Review of Literature. - to be presented at the workshop "Infrared Lasers and Millimeter Waves: The Links between Microwaves and Laser Optics", to be held at Brooks AFB, TX, on 21-22 Jan. 1997.
2. Prol, H. K.; **Pakhomov, A.G.**; Mathur, S. P.; Campbell, C.B.G. Comparison of Field Intensity Levels on the Biological Effect of Millimeter Waves at 41.34 GHz.

Second CERT Symposium on Environmental Radiation Toxicology (September 19-20, 1996, San Antonio, TX), 1996

3. **Pakhomov, A.G.**; Prol, H. K.; Mathur, S. P.; Akyel, Y., Campbell, C.B.G. Frequency and Intensity Dependence of the Millimeter-Wave Radiation Effect on Isolated Nerve Function. *Abstracts of the 18th Annual Meeting of the Bioelectromagnetics Society (June 9-14, 1996, Victoria, B.C., Canada), 1996, p. 75*

4. Pakhomova, O. N.; **Pakhomov, A. G.;** Akyel, Y. Effect of Millimeter Waves on Mutagenic And Recombinagenic Repair in Yeast. *Third International Congress of the European Bioelectromagnetics Association (February 29 - March 3, 1996, Nancy, France), 1996*

5. **Pakhomov, A.G.**; Prol, H. K.; Mathur, S. P.; Akyel, Y., Campbell, C.B.G. Frequency-Specific Effects of Low-Intensity Millimeter Waves on Isolated Nerve Function. *Third International Congress of the European Bioelectromagnetics Association (February 29 - March 3, 1996, Nancy, France), 1996.*

6. **Pakhomov, A.G.;** Prol, H. K.; Akyel, Y., Campbell, C.B.G. Frequency Dependence of Low-Level Millimeter Waves Effect on Nerve Conduction. *First CERT Symposium on Environmental Radiation Toxicology (September 21-22, 1995, San Antonio, TX), 1995*

7. Pakhomova, O. N.; **Pakhomov, A. G.;** Akyel, Y. Mutagenic and Recombinagenic Effects of Millimeter-wave Radiation. *First CERT Symposium on Environmental Radiation Toxicology (September 21-22, 1995, San Antonio, TX), 1995*

8. **Pakhomov, A.G.** "Millimeter Wavelength Radiation Effects on Nerve Function" - presented at Review and Analysis at USAMRD/WRAIR (1995) and at the 2nd Michaelson Research Conference, Kalispell, MO (1995).

9. **Pakhomov, A.G.;** Prol, H. K.; Akyel, Y., Campbell, C.B.G. Low-Level Millimeter-Wave Radiation Alters Isolated Nerve Resistance to a High-Rate Stimulation. *Abstracts of the 17th Annual Meeting of the Bioelectromagnetics Society (June 18-22, 1995, Boston, MA), 1995, p. 46.*

10. Prol, H. K.; **Pakhomov, A.G.;** Akyel, Y., Campbell, C.B.G. A Screening Assessment of Frequency-Specific Effects of Millimeter Waves on Isolated Nerve

Function. *Abstracts of the 17th Annual Meeting of Bioelectromagnetics Society (June 18-22, 1995, Boston, MA), 1995, p. 110-111*

11. **Pakhomov, A.G.;** Dubovick, B.V.; Pronkevich, A.N. Microwave Effect on Nerve Function in Various Isolated Preparations. *Abstracts of the World Congress on Medical Physics and Biomedical Engineering (21-26 August 1994, Rio de Janeiro, Brazil), 1994, Part 1, p. 67*

14. Publications and Papers Resulting from NRC Associateship Research:

a) Publications in peer-reviewed journals (all have been accepted, currently are in press):

1. **Pakhomov, A. G.;** Prol, H. K.; Mathur, S. P.; Akyel, Y.; Campbell C.B.G. Role of Field Intensity In The Biological Effectiveness of Millimeter Waves At A Resonance Frequency. *Bioelectrochemistry and Bioenergetics, 1997, in press.*
2. **Pakhomov, A. G.;** Prol, H. K.; Mathur, S. P.; Akyel, Y.; Campbell C.B.G. Search for Frequency-Specific Effects of Millimeter-Wave Radiation on Isolated Nerve Function. *Bioelectromagnetics 1997, in press.*
3. Pakhomova, O. N.; **Pakhomov, A. G.;** Akyel, Y. Effect of Millimeter Waves on UV-Induced Recombination and Mutagenesis in Yeast. *Bioelectrochemistry and Bioenergetics, 1997, in press*
4. **Pakhomov, A. G.;** Prol, H. K.; Mathur, S. P.; Akyel, Y.; Campbell C.B.G. Frequency-Specific Effects of Millimeter Wavelength Electromagnetic Radiation In Isolated Nerve. *Electro- and Magnetobiology, 1997, 16(1), 43-57, in press*

b, c) - there are no other submitted or published works

15. Patent or Copyright Applications Resulting from NRC Associateship Research

No

16. Future Position and Address and or Forwarding Address.

Situation is unclear yet. Although I have applied for a green card, there was no response so far. After the end of the tenure, my family and I may either return to Russia to (then, you can use my Russian address), or we will stay here (then, my current address will be valid). I will let you know where to send the form 1042S.

17. Appraisal of the Associateship Programs:

I value the Associateship Program very high, and appreciate all research and professional opportunities provided by this program.

NATIONAL RESEARCH COUNCIL
ASSOCIATESHIP PROGRAMS
2101 CONSTITUTION AVE, NW, TJ2114
WASHINGTON D.C. 20418

FINAL REPORT

PII Redacted

Helene van CUYCK-GANDRE [redacted] January 30, 1997

Walter Reed Army Institute of Research,
Department of Virus Disease
Washington D.C. 20307-5100

DATES OF TENURE: August 8, 1994 to February 7, 1997

TITLE OF RESEARCH PROPOSAL:

HEV detection in biological samples and sequencing of viral genome.

NAME OF RESEARCH ADVISER: Col. C. HOKE
Col. C.F. LONGER

PROFESSIONAL POST:

Specialist of Research in Biochemistry
Centre de Recherches du Service de Sante des Armees
Unite de Virologie
24 Ave des Maquis du Gresivaudan, BP 87
38702-La Tronche Cedex
France
Tel: 011 (33) 476-63-69-00
FAX: 011 (33) 476-63-69-17

PROFESSIONAL TRAVEL DURING TENURE:

In USA:

-Cincinnati, Ohio, Nov. 1994. ASTMH, 43rd annual meeting.
-Austin, Texas, July, 1995. ASV, 14th meeting.
-San Antonio, Texas. Nov. 1995. ASTMH, 44th annual meeting.
-London, Canada. July, 1996. ASV 15th meeting.
-Baltimore, Dec., 1996. ASTMH, 45th annual meeting.

Foreign meetings:

-Grenoble, France, Dec.1994, Chemistry thesis committee on Hepatitis E Virus.

-Paris, Oct. 1995. Internationnal Symposium on Enterically-Transmitted hepatitis Viruses.
-Lyon, Dec. 1995. Physician thesis committee on Hepatitis E Virus.
-Rome, Italy. April, 1996. IX triennal internationnal symposium on viral hepatitis and liver disease.
-Marseille, France. June, 1996. French Military Tropical Diseases symposium.

SEMINARS DELIVERED AT INSTITUTE

-Plant RNA viruses, WRAIR
-Comparison of the performances of different computer program to analize sequences, WRAIR

SUMMARY OF RESEARCH DURING TENURE (Significant findings:<100 words)

The genomes for an Asian (Abbottabad) and two African HEV isolates (Chad and Algeria) were sequenced and compared to HEV reference strains. The African isolates constitute a separate branch inside the Asian group. However the Chad strain has mutations common with both Aslan and Mexican strain besides its own mutations, modifying the concept of two separate genotypic branches of HEV.

The Abbottabad isolate was related to Burma strain, showing that two variants can circulate in the same country.

The population of HEV consists of different variants within the same patient and between two patients in the same outbreak .

RESEARCH IN PROGRESS

-Expression of a protein from the ORF1 part of the HEV genome
-Influence of the anti-HEV source to capture HEV

PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES

-H. van Cuyck-Gandre, J.D. Caudill, H.Y. Zhang, C.F. Longer, C. Molinie, R. Roue, R. Deloince, P. Coursaget, M. Nahor N'Gawara, Y. Buisson. PCR detection of HEV In North African fecal samples. ASTMH, 1994, Cincinnati, Ohio, USA (Poster).
-H. van Cuyck-Gandre, H.Y. Zhang, N.J. Clements, J.D. Caudill, P. Coursaget, Y. Bulsson, R.L. Warren, C.F. Longer. Sequence of hepatitis E virus (HEV) from North Africa and Pakistan: comparison with known sequences. American Society for Virology, 14th Annual meeting, July 8-12, 1995, Austin, Tx., USA (Poster)
-H. van Cuyck-Gandre, H.Y. Zhang, N.J. Clements, J.D. Caudill, P. Coursaget, Y. Bulsson, R.L. Warren, C.F. Longer. Partial sequence of HEV isolates from North Africa and Pakistan. International symposium on enterically-transmitted hepatitis viruses. HIA Val de Grace, Oct.16-17, 1995, Paris, France (oral)
-H.Y. Zhang, J. Burrous, BT Zhao, J.D. Caudill, H. van Cuyck-Gandre, R. Putnak, R.L. Warren, C.F. Longer. Baculovirus expression of the hepatitis E virus (HEV) gene segment containing the full length combined open reading frame (ORF) 2 and 3. ASTMH, Nov. 21-27, 1995, San Antonio, Tx., USA (Poster).

-H. van Cuyck-Gandre, N.J. Clements, S. J. Cohen, J.C. Caudill, R.L. Warren, C.F. Longer. 1996. Variation in the genetic population of hepatitis E virus (HEV) isolates in patients from an outbreak in Chad. IX triennal international symposium on viral hepatitis and liver disease, Rome, Italie. (Poster and oral).
-H. van Cuyck-Gandre, N.J. Clements, S. J. Cohen, J.C. Caudill, R.L. Warren, P. Coursaget, Y. Buisson, C.F. Longer. Hepatitis E Virus in Africa. French military Tropical Diseases symposium, 1996, Marseille, France.
-Van Cuyck-Gandre H., Cokman-Thomas R., Caudill J.D., Asher L. V. S., Amstrong K. L., Buisson Y., Binn L.N., and Longer C.F. Experimental african HEV infection in cynomolgus macaques ASTMH, 1996, Baltimore (oral)

PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH

Publications in pre-reviewed journals:

H. van Cuyck-Gandre, J.D. Caudill, H.Y. Zhang, C.F. Longer, C. Molinie, R. Roue, R. Deloince, P. Coursaget, M. Nahor N'Gawara, Y. Buisson. Polymerase chain reaction detection of hepatitis E virus in North African fecal samples. American Journal Tropical Medecine and Hygiene, 1996, 54, 134-135.

Books and book chapters:

H. van Cuyck-Gandre, H.Y. Zhang, N.J. Clements, J.D. Caudill, P. Coursaget, Y. Buisson, R.L. Warren, C.F. Longer. Partial sequence of HEV isolates from North Africa and Pakistan. Dans: Enterically-transmitted hepatitis viruses, Y. Buisson, P. Coursaget et A Kane Eds. La Simarre, joue-les-Tours, France. 1996, pp301-310.

Manuscript in preparation and manuscripts submitted:

-H. van Cuyck-Gandre, H.Y. Zhang, N.J. Clements, J.D. Caudill, P. Coursaget, Y. Buisson, R.L. Warren, C.F. Longer. Characterization of hepatitis E virus isolates from Algeria and Chad outbreaks by partial genome sequence. (submitted)
-van Cuyck-Gandre H., Clements N.J., Cohen S., Caudill J.D., Buisson Y., B. Innis, Warren R.L., Longer C.F. Quasi-species nature of HEV observed in replicate analysis of patient specimens. (manuscript in preparation).
-van Cuyck-Gandre H., Cokman-Thomas R., Clements N.J., Caudill J.D., Asher L. V. S., Amstrong K. L., Buisson Y., Binn L.N., and Longer C.F. Experimental african HEV infection in cynomolgus macaques (submitted).
-Coursaget P., Buisson Y., Nahor N'Gawara M., van Cuyck-Gandre H., Roue R. Role of hepatitis E virus in sporadic cases of acute and fulminant hepatitis in an endemic area (soumis a publication).

FUTURE POSITION AND ADDRESS

Adjoint au chef de l'unite de Virologie
Centre de Recherches du Service de Sante des Armees
Unite de Virologie
24 Ave des Maquis du Gresivaudan, BP 87
38702-La Tronche Cedex
France
Tel: 011 (33) 476-63-69-00 FAX: 011 (33) 476-63-69-17

APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

This program permitted to devote myself entirely to a project, to extend my knowledge on hepatitis E virus, virology and vaccine development. Opportunities in the development of the project were possible dues to the experience of Col. CF Longer and B. Innis and all those who are citated in the different manuscripts.

I appreciated to have funds for presenting the work that I have done to meetings. It was stlmulating to do the synthesis of the results and facilitate the publication work. Usually the fruits of the work are harvested after the associateship program ends. It would be desirable to authorize the participation at least at one meeting withln the year after the end of the tenure.

H. van CUYCK-GANDRE

Date: July 28, 1997

Name: Xiaoyan Zhang; ID: 9493240

Name of Laboratory: Walter Reed Army Institute of Research.

Date of tenure: April 11, 1995 - July 25, 1997.

Title of research proposal: Phospholipase stimulated metabolism in the breast cancer cells (MCF-7) and their multidrug resistant cells (MCF-7 ADR) cultures.

Name of research division: Pathology

Professional travel during tenure:

1. Attend a 4th International Conference " EICOSANOIDS & OTHER BIOACTIVE LIPIDS IN CANCER, INFLAMMATION & RADIATION INJURY" in Hong Kong (Oct. 2- 8, 1995).
2. Attend 87th Annual Meeting of American Association for Cancer Research at Washington, DC.
3. Attend the meeting " Thirteenth Annual Meeting on Oncogenes" At Hood college, MD.

Summary of Research during tenure:

Proliferation and inhibition of cell growth in cultures of MCF-7 WT and MCF-7 ADR were examined with standard [methyl-^{3}H]-thymidine incorporation. Some inhibitors and heteropolyanions are very effective to both breast cancer cells and some of them only effect on one cell line. We evaluated the toxicity of the inhibitors and heteropolyanion using colony assay with light density human bone marrow cells. The heteropolyanion drugs as free-radical scavenger suggest a role in modification of arachidonate metabolism. We studied arachidonic metabolite distributions with/without treatment of inhibitors or heteropolyanions and tried to understand the death of the cells by cell cycle studies..

Research in progress:

The toxicity of a heteropolyanion was examined with animal model (mice). After injection or admission of the drug, the organs were digested. The content of tungsten in different organs will be measured with ICP. The following questions will be answered. The arachidonic acid metabolism experiments were done and the samples are waiting for HPLC analysis.

Presentations at scientific meeting:

1. Xiao yan Zhang, Charles P. Chang, R. Tran, Julie Weitz, James L. Mulshine, Marti Jett, Meeting Abstract (1996) in 87th annual meeting of AACR.
2. Y. Wang, X. Zhang, J. Weitz, M. Jett., Meeting Abstract (1996) in ASBMB/ASIP/AAI Joint Meeting.

FINAL REPORT

1. 5/24/97

2. **Michael Bray, M.D.**

3. Virology Division, USAMRIID, Fort Detrick, Frederick, MD 21702-5011

4. 5/13/95 -- 3/31/97

5. Prophylaxis and treatment of filovirus infections

6. Dr. John Huggins

7. Not on leave.

8. No offices held.

9. Travel in U.S.:

   2/96 American Association for the Advancement of Science, Baltimore, MD
   12/96 American Society for Tropical Medicine and Hygiene, Baltimore, MD

   Foreign travel:

   11/95 W.H.O. Regional Conference on Emerging Infectious Diseases, Cairo, Egypt
   9/96 International Symposium on Ebola Virus Research, Antwerp, Belgium (not funded by NRC)

10. None.

11. While an NRC senior associate, I adapted Ebola Zaire virus to adult, immuno-competent mice through serial passage. The mouse-adapted virus is uniformly lethal for mature BALB/c, C57Bl6 and ICR mice. The infection in mice resembles Ebola hemorrhagic fever of primates. Animals die 5-6 days after intraperitoneal inoculation of virus; the liver and spleen are major sites of injury. We have used this mouse model to demonstrate the *in vivo* efficacy of antiviral drugs which have *in vitro* activity against Ebola, and have been able to protect mice treated 2-3 days following viral challenge.

12. Continuation of above, investigating the pathogenesis of Ebola infection in mice and the efficacy of antiviral drugs for Ebola infection of primates..

13. Presentations:

    9/96 International Symposium on Ebola Virus Research, Antwerp, Belgium:

    A mouse model for evaluation of Ebola prophylaxis and therapy. Mike Bray, Kelly Davis, Tom Geisbert, Connie Schmaljohn, John Huggins. USAMRIID, Fort Detrick, Frederick, MD, USA.

12/96 American Society for Tropical Medicine and Hygiene, Baltimore, MD:

Adaptation of Ebola Zaire virus to adult, immunocompetent mice. Mike Bray, Kelly Davis, Tom Geisbert, Connie Schmaljohn, John Huggins. USAMRIID, Fort Detrick, Frederick, MD, USA.

14. Manuscripts submitted:

a. None
b. None
c. Submitted to the Journal of Infectious Diseases:

A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. Mike Bray, Kelly Davis, Tom Geisbert, Connie Schmaljohn, John Huggins. USAMRIID, Fort Detrick, Frederick, MD, USA

15. None.

16. Future position:

Contract employee, Virology Division, USAMRIID, Fort Detrick, Frederick, MD 21702-5011

17. Provided a unique opportunity for research on Ebola virus.

FINAL REPORT

(1) **Date:** 1/06/96

(2) **Name:** Gabriela Alicia Canziani

(3) **Laboratory:**
Department of Immunology and Molecular Biology
Toxinology Division
USAMRIID
1425 Porter Street
Fort Detrick, Md 21702-5011
(MCMR-UIZ-A)

(4) **Dates of Tenure:** 2/4/94 to 1/21/96

(5) **Title of Research Project:**
"Characterization of Attenuated mutant Staphylococcal enterotoxin candidate vaccine"

(6) **Research Adviser:** Robert G. Ulrich, Ph.D.

(7) **Leave from a professional post:**
Research Associate Position at Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Buenos Aires, Argentina. Until July1996.

(8) N/A

(9) **Programmatic Travel during tenure:**

- Antibody-Based Therapeutics, the latest Clinical Trial and Application Strategies. June 23-24, 1994. Washington, DC.

-Fourth Annual BIAsymposium> Biomolecular Interaction Analysis. September 26-28, 1994. Baltimore, Maryland.

- Keystone Symposium on: "Control and Manipulation of the Immune

- Keystone Symposium on: "Control and Manipulation of the Immune Response" March 16-22, 1995; Taos, New Mexico.

- 9th International Congress of Immunology, July 23-29, 1995, San Francisco, California.

- Joint Meeting of FASEB Meeting, June 1-6, 1996. New Orleans.

- 6th Annual BIA Symposium, October 8-10,1996. Washington, DC

(10) **Seminars or Lectures delivered at Universities and Institutes**

-Mode of binding of Staphylococcal enterotoxins to Major Histocompatibility Complex Class II, a biosensor study. At the Rheumatology Division, University of Pennsylvania, Department of Medicine (909 Stellar-Chance Laboratories. 25 November 1996

-Bacterial Superantigens binding to Major Histocompatibility Complex Class II receptors Invited to the Research Seminars in Immunology organized by Dr. A.J. Russo at the Science Dept. Mount Saint Mary's College, MD. 21 November 1996.

-Cell surface complexes of HLA-DR1 and Bacterial superantigens or CD4 are more stable than trimolecular complexes with T-cell antigen receptors. Invited to participate in the Superantigen Workshop, Chairman : J. Kappler. 9th International Congress of Immunology, July 23-29, 1995.

(11) **Summary of Research during Tenure:**

We determined the kinetic stability of the trimer formed by bacterial superantigen Staphylococcal enterotoxin A (SEA) bound to Major Histocompatibility complex class II receptors and the T-cell receptor using a set of monoclonal antibodies produced and characterized in the lab. Using biosensor technology we determined affinity constants and free energies of binding ($\Delta\Delta Gs$) of wild type SEB superantigen and mutants with HLA-DR1 (human MHC classII receptor).
Overall, these biochemical and biophysical approaches have improved our

therapeutic strategies by elucidating how surface epitopes of Staphylococcal enterotoxins A and B interact with their natural receptors and how these interactions are transduced into signalling of T-cells to proliferate and to produce cytokines responsible for toxicity.

(12) **Research in progress:**

The main goal is to complete a publication including the kinetic analysis of Staphylococcal enterotoxin B binding to its natural receptor in the immune system: the Major Histocompatibility Complex class II, using biosensor, flow cytometry and ELISA techniques. The major goal is to handle rapidly the binding properties of mutants of bacterial toxins which are vaccine candidates for the research programs developed in this laboratory. This will be a methodology publication.

(13) **Presentations at Scientific Meetings:**

-Canziani, G.A.; Crosland, R. (1995) Fluorescent sphere tagging of Botulinum A toxin bound to target tissues: A labelling technique. Meeting of the American Society of Neurology. San Diego, November 15-18, 1995

-Bavari, S; Anderson, N; Canziani, G.A.; Ulrich, R.G.(1995) Kinetics of bacterial superantigen binding and T-cell recognition. 9th International Congress of Immunology Sponsors: The American Association of Immunologists, The International Union of Immunological Societies, July 23-29, 1995.

-Canziani, G.A.; Callahan, K.; Stiles, B.G.; Bavari, S.; Ulrich, R.G. (1995) Cell surface complexes of HLA-DR1 and Bacterial superantigens or CD4 are more stable than trimolecular complexes with T-cell antigen receptors. 9th International Congress of Immunology, July 23-29, 1995. Sponsors: The American Association of Immunologists, The International Union of Immunological Societies.

-Canziani, G.A.; Viskatis, L; Langforf, R. Vidal, J.C.; Kaiser, I. (1994) Crotoxin: A diaphragm specific toxin? 8th World Meeting of the International Society on Toxinology (IST), Tel-Aviv, Israel, Oct 1994.

(16) **Manuscripts in preparation, manuscripts submitted:**

-Canziani, G.A.; Bavari, S; Ulrich, R.A Modification of residues involved in binding of bacterial superantigen Staphylococcal enterotoxin B to HLA-DR1 and stability of the complex measured by surface plasmon resonance. In preparation

-Canziani, G.A., Callahan, Stiles, B.; K; Bavari, S; Ulrich, R.A. (1996) The kinetic pathway of DR-dependent T-cell signalling by the bacterial superantigen staphylococcal enterotoxin A. To be submitted.

-Crosland, R. and Canziani, G.A. (1996) Localization of Botulinum toxin A binding to cholinergic nerve endings with fluorescent latex spheres. Submitted.

(15) N/A

(16) **Future Position:**
Appointed Operator/Manager of Biosensor-Molecular Interaction Analysis Core Facility
Rheumatology Division
University of Pennsylvania School of Medicine
913 Stellar Chance Laboratories (Biomedical Research Building)
422 Curie Boulevard
Philadelphia, PA 19104-6100
Phone: (215) 662-2353
FAX: (215) 349-5572

(17) **Appraisal of Associateship Programs:**

The NRC Program allowed me to work in a collaborative and interactive environment has been positive at this stage of my career. The Award and the laboratory where I have worked for the past 35 months brought together a variety of facilities (cutting-edge technologies) and resources, such as information and professional contacts.

The participation in Scientific Meetings supported by the Programmatic Travel enabled professional encounters with specialists in the specific area of research and other areas which opened future research opportunities.
NRC is a prestigious Program and as such has provided the opportunity to generate interest of specialists in fields of expertise different from that of the Associateship.

My Adviser played a major role in making benefits apparent to me which are otherwise would have been difficult to grasp when my attention was focused on laboratory work and acquaintance with new concepts and techniques. In my case, the role of the Advisor was pivotal for my success. Besides attending to my research program Dr. Robert G. Ulrich promoted full use of the benefits offered by the NRC Associateship Programs, kept me informed permanently about meetings and conferences, and encouraged me to make full use of the Programmatic Travel benefits.

cc: Dr. Carol Linden
Dr. Robert Ulrich

National Research Council
Associateship Program
2101 Constitution Avenue, NW
Washington, DC 20418

OCT 24 1996

FINAL REPORT

(1) **DATE** 10/15/96

(2) **NAME** Brett M. Connolly, PhD
PII Redacted [redacted]

(3) **LABORATORY** US Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick MD

(4) **DATES OF TENURE** 10/18/93–10/18/96

(5) **TITLE OF RESEARCH PROJECT** Pathogenesis of Filoviruses in Animal Models using Refined Techniques of Immunohistochemistry and Electron Microscopy

(6) **RESEARCH ADVISOR'S NAME** Peter B. Jahrling, PhD

(7) **ARE YOU ON LEAVE FROM A PROFESSIONAL POST?** No

(8) **INTERNATIONAL POSITIONS HELD DURING TENURE** N/A

(9) **PROGRAMMATIC TRAVEL DURING TENURE** None

(10) **SCIENTIFIC SEMINARS, MEETINGS, AND/OR CONSULTATIONS** None

(11) **SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES**
Centers for Disease Control and Prevention 9/16/96
University of Delaware 10/14/96

(12) **MEETINGS ATTENDED BY SPECIFIC INVITATION** None

(13) **TEACHING, IF ANY, AS AN ASSOCIATE** N/A

(14) **WORK IN PROGRESS** Drafting results of Ebola pathogenesis study

(15) **SUMMARY OF RESEARCH DURING TENURE** Ebola–Zaire virus was adapted to produce lethal infection in strain 13 guinea pigs. This guinea pig model was used to investigate the pathologic events leading to death in a sequential sacrifice study using immunohistochemical, *in situ* hybridization and electron microscopic techniques. Cells of the mononuclear phagocytic system (MPS),including macrophages, Kupffer cells and fixed tissue histiocytes, were identified as the early and sustained cellular targets of Ebola virus. The distribution of lesions, hematologic profiles, and increases in certain serum biochemical enzymes mirrored those reported for naturally infected humans and experimentally infected nonhuman primates.

(16) **PUBLICATIONS AND PAPERS RESULTING FROM RESEARCH AS AN ASSOCIATE**

Pathogenesis of experimental Ebola Virus Infection in Guinea Pigs. Connolly BM, Steele KE, Davis KJ, Geisbert TW, Kell WM, Jaax NJ, Jahrling PB. Submitted to Journal of Infectious Diseases.

Pathology of Experimental Ebola Virus Infection in African Green Monkeys: Involvement of Fibroblastic Reticular Cells. Davis KJ, Anderson AO, Geisbert TW, Steele KE, Geisbert JB, Vogel P, Connolly BM, Huggins JW, Jahrling PB, Jaax NJ. Submitted to Archives of Pathology

Distribution of Ebola virus Zaire (Mayinga) in tissues of experimentally infected guinea pigs. Vogel P, Connolly B, Abplanalp D, Geisbert JB, Kell WM, Jahling PB, Jaax NJ. Manuscript in preparation.

(17) **PATENTS APPLIED FOR AS A RESULT OF RESEARCH AS AN ASSOCIATE** N/A

(18) [redacted]

PII Redacted

(19) **APPRAISAL OF THE ASSOCIATESHIP PROGRAMS**

National Research Council

Final Report

5-28-97

Dr. Kevin J. Gilligan
USAMRIID, Ft. Detrick
Frederick MD 21702-5011

"Expression of Ebola virus proteins by poxvirus-based expression vectors to define the antigenicity of individual viral components".

Dr. Kevin Anderson-Advisor

Meetings: "Advances in Vaccines" - Alexandria, VA February, 1995.
"14th Annual ASV Meeting" - Austin, TX July, 1995.
"Molecular Approaches to the Control of Infectious Diseases"- Cold Spring Harbor, NY Sept., 1996.

"Xth International Congress of Virology" - Jerusalem, Israel 1996.

Summary of Research:

I have constructed recombinant vaccinia viruses that express Ebola virus proteins and used them to examine their efficacy in a series of vaccination/challenge experiments. I have found that one of my constructs, rVV/GP, will completely protect guinea pigs against lethal challenge when administered in two doses. I have also found that the immunity induced by this rVV is reduced when co-administered with rVV/SGP or rVV/VP40. Taken together, these experiments have proven the efficacy of using Ebola virus GP to induce protective immunity and also has provided insight into how Ebola virus infection induces immune dysfunction and causes severe disease.

Research in Progress:

I also have evidence that rVV/VP24 may induce a measure of immunity and experiments are in progress to provide data to support this observation. In addition, I am currently involved in studies which are designed to identify the nature of the protective immune response induced by my vaccine constructs in order to provide an understanding of how Ebola virus immunity is achieved and how this immunity fails during a natural infection.

Oral Presentations:

14th Annual ASV Meeting - Austin, TX July, 1995.
Molecular Approaches to the Control of Infectious Diseases- Cold Spring Harbor, NY Sept., 1996

Xth International Congress of Virology- Jerusalem, Israel 1996.

Publications:

Gilligan, K. J., Geisbert, J. G., Jahrling, P. B., Anderson, K. (1997) Vaccines 97 Cold Spring Harbor Press pp 87-92.

Gilligan, K. J., Giesbert, J. G., Wasieloski, L., Jahrling, P. B. and Anderson, K. Manuscript submitted.

Gilligan, K. J., Geisbert, J. G., Jahrling, P. J. and Anderson, K. Manuscript in preparation.

Forwarding Address:

Division of Diagnostics
Bldg. 1425, USAMRIID
Ft. Detrick
Frederick, MD 21702-5011

Appraisal of Program:

All in all, I was very happy with the program. I found the work very gratifying and the associateship was managed well by the NRC. If I have one criticism, it is with the timeframe in which the travel money can be used. In my own personal case, the work I have completed up to now is very important and needs to be presented at international meetings. However, my tenureship is ending before the meeting I wish to present it at is scheduled. I think it would be desirable to have a grace period of two to three months whereby NRC travel money can be carried over after the termination of the fellowship.

9424840

Final Report for National Research Council Associateship Programs

Date: May 15, 1997

Name: Dr. Michael Hevey

Laboratory: USAMRIID

Dates of Tenure: July 24, 1994 - May 31, 1997

Title of Research Proposal: Examination of the Role of Cell Mediated and Humoral Immune Responses to Filovirus Antigens

Research Advisor: Dr. Alan Schmaljohn

Are you on leave from a professional post? No

Professional society offices held during tenure: None

Professional Travel During Tenure:

American Society for Virology Meeting, University of Texas, Austin, Texas, July 8-12, 1995
American Society for Virology Meeting, University of Western Ontario, London, Ontario, Canada, July 13-17, 1996
Molecular Approaches to the Control of Infectious Diseases meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, September 9-13, 1996

Seminars or lectures delivered at universities and/or institutes: None

Summary of Research During Tenure:

Basic concepts relevant to Marburg virus (MBG) vaccine development were explored by attempting to define minimal requirements for protective immunity in an animal model system. Because the protective antigens of MBG were unknown, the three most abundant virion proteins were chosen for expression in a baculovirus vector and testing for protective efficacy. Using a guinea pig model system it was demonstrated that a subunit vaccine consisting of MBG glycoprotein was sufficient to induce an immune response that protected the animals from a lethal challenge of a homologous isolate but not a heterologous isolate. However, a more "classical" vaccine consisting of inactivated virion in adjuvant was demonstrated to protect guinea pigs from challenge with either a homologous or heterologous isolate. Furthermore, antibody alone was determined to be protective in the guinea pig model system, through passive transfer experiments. Neither of the other two virion proteins examined (Nucleoprotein and VP40) protected animals when used as a subunit vaccine. In addition, monoclonal antibodies (MAbs) were made against whole virion. Several of the resulting MAbs were characterized with respect to their specificity, isotype, and ability to compete with each other for binding antigen. In addition, several of the MAbs were examined for biological activity in neutralization assays, chromium release assay, and ability to protect guinea pigs via passive transfer. The epitopes of six of the MAbs which demonstrated neutralization activity *in vitro* and/or the ability to protect guinea pigs via passive transfer were determined to a resolution of 9 amino acids on the MBG GP protein. Taken together this body of work has demonstrated that in the guinea pig model system MBG GP is a protective antigen, and that antibodies to GP are sufficient for protection. Future experiments will delineate how these observations are relevant in a non-human primate model.

Research in Progress:

Current studies are designed to identify a surrogate marker for protection in the guinea pig model. In addition, the role of cell mediated immunity in infection is just beginning. The MBG genes have been cloned and expressed in an alphavirus replicon and animals immunized to determine if viral antigens other than GP are protective. Finally, progress is being made toward testing potential vaccine candidates in non-human primates.

Presentations at scientific meetings:

CHARACTERIZATION OF MARBURG PROTEINS EXPRESSED IN Sf-9 CELLS USING A BACULOVIRUS SYSTEM

M. Hevey and A. Schmaljohn
Virology Division, USAMRIID, Ft.Detrick, Frederick, MD

The family *Filoviridae* contains two members, Marburg (MBG) and Ebola viruses, which can cause acute hemorrhagic fevers with high mortality rates. Because no vaccines or effective treatments for filovirus infections exist, we initiated vaccine development for MBG by attempting to define minimal requirements for protective immunity. Three major components of the virion, a single glycoprotein (GP), nucleoprotein (NP), and a matrix protein (VP40), were subcloned into baculovirus expression vectors. In addition, two mutants of the GP gene were constructed, consisting of carboxy terminal deletions of two sizes. Analyses of protein expression in recombinant baculovirus infected cells revealed the following: NP, GP, and the less truncated GP mutant were expressed at high levels. The VP40 protein was expressed at a lower level, while the GP mutant with a larger C-terminal deletion failed to yield an authentic protein. These expressed proteins can be used to evaluate the immune response to MBG viral proteins, to help identify surrogate markers of immunity, and to generate reagents for use in examining the life cycle of MBG virus.

Presented at: American Society for Virology Meeting, University of Texas, Austin, Texas

MARBURG VIRUS VACCINES DERIVED FROM PROTEINS EXPRESSED IN Sf-9 CELLS

M. Hevey, D. Negley and A. Schmaljohn
Virology Division, USAMRIID, Ft.Detrick, Frederick, MD

The family *Filoviridae* contains two members, Marburg (MBG) and Ebola viruses, which can cause acute hemorrhagic fevers with high mortality rates. Because no vaccines or effective treatments for filovirus infections exist, we initiated vaccine development for MBG by attempting to define requirements for protective immunity. As part of this effort, three major components of the virion, the glycoprotein (GP), nucleoprotein (NP), and a matrix protein (VP40), were subcloned into baculovirus expression vectors. In addition, a mutant constructed from the GP gene bore a carboxy terminal deletion resulting in elimination of the putative transmembrane domain (TM-). The resulting expressed proteins were used to immunize guinea pigs (strain 13), which were later challenged with either a homologous or heterologous strain of MBG. Results indicated that animals immunized with GP as well as TM- seroconverted, as measured by ELISA, after two inoculations one month apart. No detectable antibodies to MBG were observed in animals that received either NP or VP40 after two inoculations. Challenge studies revealed that TM- is sufficient for protection from infection with a homologous MBG strain, but not from a heterologous strain of MBG. Protective efficacies of the remaining expressed proteins are currently being evaluated.

Presented at: American Society for Virology Meeting, University of Western Ontario, London, Ontario, Canada

IDENTIFICATION OF PROTECTIVE ANTIGENS FROM MARBURG VIRUS AND EVALUATION OF A POTENTIAL SUBUNIT VACCINE

Hevey, M., Negley, D., and Schmaljohn A., Virology Division, United States Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, MD 21702

Marburg virus (MBG), a prototypical member of the *Filovirus* genus, causes severe hemorrhagic fever with high mortality rates in humans. There is no available vaccine and no known effective treatment for victims of either MBG or the related filovirus, Ebola virus. Relatively little is known about immune responses to MBG: it is not known which viral antigens can elicit resistance to disease, nor by what mechanisms such protection occurs. To begin defining protective antigens, we cloned and expressed MBG genes by using recombinant baculoviruses. A 5% truncation of the carboxyl terminus of the glycoprotein (GP) facilitated synthesis and secretion of a soluble GP. This antigen was used in animal immunization experiments, from which we determined that the viral GP served as a protective immunogen in the guinea pig model system. Greater protection was observed against the MBG isolate from which the GP gene was derived than from an antigenically distinct MBG isolate. We further determined that polyclonal antibodies alone were sufficient to passively confer protection in nonimmune guinea pigs given an otherwise lethal dose of MBG. In addition, a subset of monoclonal antibodies that reacted with MBG GP was identified that neutralized MBG *in vitro*. One of these monoclones protected non-immune animals in passive transfer studies. The remaining antibodies are currently being evaluated for their protective efficacy *in vivo*. Finally, the region in which the neutralizing monoclonal antibodies binds was identified in the central region of GP, and corresponds to a hypervariable region of the peptide sequence. Collectively, these results indicate the likely utility of GP as a protective immunogen, but also raise caveats about antigenic variability among filoviruses.

Presented at: Molecular Approaches to the Control of Infectious Diseases meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Publications and Papers resulting from NRC associateship research:

Hevey, M., Negley, D., Geisbert, J., Jahrling, P., Schmaljohn, A. Recombinant Marburg virus glycoprotein subunit vaccine protects guinea pigs from lethal infection. In: Brown, F., Burton, D., Doherty, P., Mekalanos, J., Norrby, E. (eds). 1997. Vaccines 97. Cold Spring Harbor Press. Cold Spring Harbor, NY. 93-98.

Hevey, M., Negley, D., Geisbert, J., Jahrling, P., Schmaljohn, A. Prototypic Marburg virus vaccine containing glycoprotein subunit can protect guinea pigs from lethal infection. (in preparation)

Nardin, A., Sutherland, W. M., Hevey, M., Schmaljohn, A., Taylor, R.P. Quantitative studies of heteropolymer-mediated binding of inactivated Marburg virus to the complement receptor on primate erythrocytes. (submitted)

Patent or copyright applications resulting from NRC associateship research: None

Future Position and Forwarding address:

Contractor through Geo-Centers, Inc. at USAMRIID
USAMRIID
Virology Division
Fort Detrick
Frederick, MD 21702

Appraisal of the Associateship Program:

Throughout my tenure as an NRC Associate I have had only positive experiences. The people administering the program have been very helpful, the procedures for travel were efficient, and my interactions with my research advisor as well as other scientists at USAMRIID were productive and friendly.

NRC ASSOCIATESHIP FINAL REPORT

1. May 15, 1997
2. **Name:** Daniel Frederick Muldoon (**NRC I.D. Number:** Unknown)
3. **Center and Location**: U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Ft. Detrick, Frederick, Maryland
4. **Dates of Tenure:** June 4, 1995-June 3, 1997
5. **Title of Research Proposal:** "Characterization of ceramide modulation of ricin toxicity *in vitro.*"
6. **NRC Research Adviser:** Robert B. Wellner, Ph.D.
7. **On Leave From A Professional Post?** No.
8. **Professional Society Offices Held During Tenure:** None
9. **Professional Travel During Tenure:** None
10. **Seminars or Lectures Delivered at Universities and/or Institutes:** USAMRIID, May 22, 1997
11. **Summary of Research During Tenure:** I examined the modulating effects of selected drugs on the cytotoxicity of ricin, *Pseudomonas* toxin and similar protein toxins in Chinese hamster ovary cells. Primarily I studied the sphingolipid metabolite ceramide and its interaction with brefeldin A and ilimaquinone against the toxins. Since these toxins kill cells by inhibiting protein synthesis, I used inhibition of protein synthesis (measured by amino acid uptake) as a measure of cytotoxicity. The goal was to identify drugs that could be used to protect against poisonings with protein toxins such as ricin.

12. **Research in Progress:** Completing the experiments for the project: studying the effects of ceramide on the kinetics of ricin toxicity (*i.e.*, determining the effect, if any, of ceramide on the time course of protein-synthesis inhibition).

13. **Presentations at Scientific Meetings or Conferences:** None

14. **Publications and Papers Resulting from NRC Associateship Research:** None. (A manuscript based on my NRC research is in preparation.)

15. **Patent or Copyright Applications Resulting From NRC Associateship Research:** None

16. **Future Position and Address and/or Forwarding Address:**

PII Redacted [redacted] (No new position to date).

17. **Appraisal of the Associateship Programs:** Dr. Wellner, my Research Adviser, was very helpful to me, and I learned much from being at USAMRIID. I was able to conduct research beyond the scope of what I learned in graduate school. Associateships are known for providing opportunities in respected federal laboratories; in my case, it was interesting to work in a laboratory whose endeavors have brought it fame. I really enjoyed being spared the necessity of taking the time to secure extramural funding. The generous stipends also are a definite plus. However, I found the procedure of making estimated tax payments to be much more of a headache than I had anticipated. If it were possible to withhold taxes from Associates who are U.S. citizens, it would add another advantage to a program that already has much to offer.

Final Report on Tenure

15 October 1996

Mary Alice Woody
USAMRIID, Toxinology Division
1425 Fort Detrick
Frederick, MD 21702

Dates of tenure: 21 Sept. 1994 to 20 Oct. 1996

Project title: Biological activities and vaccine potentials of mutant proteins of staphylococcal enterotoxin B (SEB) in an lipopolysaccharide-sensitized mouse model and in vitro assays.

Research advisor: Dr. Bradley G. Stiles

I was not on leave from a professional post during tenure.

Items (8), (12), (13), and (17): N/A

Programmatic travel and meetings during tenure:

American Society for Microbiology (ASM) General Meeting 21-25 May 1995, Washington, D. C.: Attended sessions on staphylococcal enterotoxins.

International Society of Toxinology (IST) meeting, 31 July 1995 to 4 August 1995, Frederick MD: Attended sessions on microbial toxins which included SEs and TSST-1; also attended other sessions. Acted as co-chair of one of the microbial toxin sessions. Presented poster on work with SEB mutant proteins.

Protein Folding and Design: An International Conference, 23-26 April 1996, National Institutes of Health, Bethesda, MD: Attended sessions and viewed posters 26 April 1996.

ASM General Meeting, 19-23 May 1996, New Orleans, LA: Presented poster on research with additional SEB mutant proteins and attended sessions on staphylococcal enterotoxins.

Work in progress:

The research with SEB mutant proteins has reached a conclusion, and the second manuscript from this work is nearing submission for peer review.

The secondary project on epitope-mapping of SEA with monoclonal antibodies will be finished by my research advisor, Dr. Stiles. The reactivity of one monoclonal antibody with the far carboxyl terminal of SEA has been studied recently with synthetic peptides containing SEA or SED sequences. The remaining experiments will involve reactivity of this monoclonal antibody with a synthetic SEE peptide. Depending on the results of these experiments, we may prepare a short manuscript for publication in a peer-reviewed journal.

Summary of research during tenure:

Mutant proteins of staphylococcal enterotoxin B (SEB) were examined for vaccine potentials. N23K, Q43P, F44P, F44S, and L45R were not lethal in lipopolysaccharide-sensitized mice and induced lower serum cytokines levels than SEB, but retained common epitopes with SEB. Immunization of mice with any of the proteins produced similar anti-SEB titers and protection against a SEB challenge that killed naive animals. Sera from immunized mice inhibited SEB-induced naive murine splenocyte proliferation in vitro. Q43P, F44P, and L45R weakly stimulated human-T-cell proliferation. Unlike the other proteins, L45R did not cause T-cell anergy.

Presentations and publications:

Poster, IST meeting, 1-2 Aug. 1995: M. A. Woody, T. Krakauer, J. Bill, and B. G. Stiles. Biological Effects and Vaccine Potential of Staphylococcal Enterotoxin B Mutants: N23K and F44S.

Poster, ASM meeting, 21 May 1996: M. A. Woody, T. Krakauer, and B. G. Stiles. Staphylococcal Enterotoxin B Mutants (N23K and F44S): Biological Effects and Vaccine Potential in a Mouse Model.

Paper accepted: Woody MA, Krakauer T, Stiles BG. Staphylococcal enterotoxin B mutants (N23K and F44S): biological effects and vaccine potential in a mouse model. Vaccine: In press.

Paper prepared for submission to the *Journal of Infectious Diseases*: M. A. Woody, T. Krakauer, R. G. Ulrich, and B. G. Stiles. Differential Immune Responses to Staphylococcal Enterotoxin B Mutations Encompassing Adjacent Residues in a Hydrophobic Loop Dominating the Interface with

Major Histocompatibility Complex Class II Receptors.

Informal seminar presented, 2 October 1996 at the National Cancer Institute, Frederick, MD: M. A. Woody, T. Krakauer, R. G. Ulrich, and B. G. Stiles. Differential Immune Responses to Staphylococcal Enterotoxin B Mutations Encompassing Adjacent Residues in a Hydrophobic Loop Dominating the Interface with Major Histocompatibility Complex Class II Receptors.

Future position and address:

Postdoctoral fellowship, initially funded by Genzyme, in the laboratory of Dr. William J. Murphy, NCI, Frederick, MD.

I can be reached at my home address:

PII Redacted

[redacted]

Appraisal of the associateship program:

This was an enjoyable and rewarding experience. I have learned a great deal in technique and theory while at USAMRIID, and have made some valuable professional contacts. One aspect of the program that I appreciated is that there are readily available funds for travel to scientific meetings. My only regret is that, because of budgetary considerations at USAMRIID, I will not have a third year as an associate, but that is a problem that the NRC program can not remedy. I hope that funding of the various participating federal agencies stabilizes so that the associateship program can continue. I will be recommending the program to others.

FINAL REPORT
To The National Research Council Associateship Programs

1. Date: July 14, 1997

2. Name: William D. Korte

3. Laboratory: US Army Medical Research Institute of Chemical Defense
   3100 Ricketts Point Rd.
   Aberdeen Proving Ground, MD 21010-5425

4. Dates of Tenure: October 1, 1996 to July 31, 1997

5. Title of Research Proposal: The Development and Evaluation of LC-MS, CE, and FIA Methods for the Detection of Sulfur Mustard Derivatives at Low Concentrations in Biological Samples

6. Research Advisor: Dr. Ming L. Shih

7. On Leave from: Professor of Chemistry
   Department of Chemistry
   California State University, Chico
   Chico, California 95929

8. Professional Society Offices Held During Tenure: None

9. Professional Travel During Tenure:

   American Chemical Society, National Meeting
   San Francisco, California
   April 12 to April 17, 1997

10. Seminars or Lectures Delivered: None.

11. Summary of Research During Tenure:

The sulfur mustard amino acid adducts, hydroxyethylthioethylvaline and hydroxyethylthioethylhistidine, were chemically synthesized by a sequence of chemical reactions. A gas chromatography-mass spectroscopy method was evaluated for the determination of hydroxyethylthioethylvaline obtained from the terminal amino group cleavage of hemoglobin that had been exposed to and reacted with the toxic agent sulfur mustard. The stability and chemical reactions of 1,3,4,6-tetrachloro-7,8-diphenyl-2,5-diimino glycoluril, a potentially useful active ingredient in a topical skin protectant against sulfur mustard, were explored.

12. Research in Progress: I am completing studies of the glycoluril for incorporation into a manuscript for publication (see below).

13. Presentations at Meetings or Conferences: No presentations based on the work listed in Item 11 were made. However, a poster "Multicomponent Spectroscopic Assay for Hemoglobin ..." based on prior work was presented at the American Chemical Society, National Meeting, in San Francisco, CA, on April 13, 1997.

14. Publications and Papers:

    Manuscript in Preparation. "Analysis, Stability, and Some Reactions of 1,3,4,6-Tetrachloro-7,8-diphenyl-2,5-diimino glycoluril, a Potential Candidate for an Active Ingredient in a Topical Skin Protectant Against Sulfur Mustard."

    Coauthors, Dr. Ming L. Shih, J. Richard Smith, and Connie Clark.

15. Patent or Copyright Applications: None

16. Future Position (same as Item 7): Professor of Chemistry
    Department of Chemistry
    California State University, Chico
    Chico, California 95929

17. Appraisal of the Associateship Programs:

    The Associateship Program was instrumental in optimizing the utilization of my Sabbatical Leave for scientific exploration and discovery. My hope would be that my publications and expertise shared both during and subsequent to my tenure with the MRICD satisfactorily compensate for the generous support that I received. I am very grateful to the NRC, the US Army MRICD and my Advisor, Dr. Ming Shih, for this opportunity.

    My only suggestion for improvement in the Associates Program would be in the handling of travel accounting. The detail of accounting reports seems unnecessary. If the Associate receives many thousands of dollars in their stipend, it seems inconsistent that they would have to report how many pennies they spent for breakfast at a scientific meeting. A per diem for food and miscellaneous expenses should be sufficient.

NATIONAL RESEARCH COUNCIL
ASSOCIATESHIP PROGRAMS
2101 CONSTITUTION AVENUE, NW, TJ2114
WASHINGTON, D.C. 20418

FINAL REPORT

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(1) Date: September 24, 1997

(2) Name: Steven F. Lewis, Ph.D.; NRC Associateship I.D. Number:

(3) Name of Laboratory or Center and Location: U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, MA 01760

(4) Dates of Tenure: Split Tenure; First Half: May 16, 1996 - September 15, 1996; Second Half: May 16, 1997 - September 15, 1997.

(5) Title of Reserch Proposal: Effects of Caffeine on Rate of Muscle Fatigue and Recovery During Dynamic Leg Exercise

(6) Name of Research Adviser: Harris R. Lieberman, Ph.D.

(7) N/A

(8) N/A

(9) Professional Travel During Tenure: American College of Sports Medicine, Annual Meeting, Denver, CO, May 27 - 31,1997

(10) Seminars or Lectures Delivered at Universities/Institutes

"A New Approach to Studying Muscle Fatigue During Dynamic Exercise in Humans"; International Symposium on Exercise in Prevention, Diagnosis and Therapy of Metabolic Disorders, Polish Academy of Sciences, Warsaw, Poland, September 25-27, 1996 (between first and second halves of tenure)

"A New Approach to Studying Muscle Fatigue and Factors Affecting Exercise Performance in Humans"; NeuroMuscular Research Center, Boston University, Boston, MA, November 26, 1996 (between first and second halves of tenure)

"A New Approach to Studying How Ergogenic Aids Affect Muscle Fatigue and Exercise Performance"; National Institutes of Health, Annual Meeting of Clinical Research Center Directors, U.S. Army Research Institute of Environmental Medicine, Natick, MA, June 26-27, 1997

(11) Summary of Research During Tenure

Dr. Lewis' work during his NRC Senior Associateship included preparation of a detailed protocol to study the effects of caffeine on human physical and mental performance (see Research in Progress below), preparation of an invited review article using the novel knee extensor muscle fatigue model he developed in collaboration with Dr. Charles Fulco of USARIEM, completion of a USARIEM research project on the ergogenic effects of a high carbohydrate diet and carbohydrate dietary supplements on knee extensor muscle performance and presentation of related research findings at scientific meetings.

(12) Research in Progress

A research protocol to study the effect of caffeine on muscle fatigue, recovery and repeat performance following exhaustive exercise has been written and - pending approval by USARIEM's Human Use Review Committee and Scientific Review Committee and by the Boston University Charles River Campus Institutional Review Board - is scheduled to begin during Fall, 1997. The protocol is based on the dynamic knee extension model exercise model developed jointly between USARIEM and Boston University

(13) Presentations at Scientific Meetings or Conferences

Posters presented at Annual Meeting of the American College of Sports Medicine, Denver, Colorado, May 27-31, 1997

Lewis SF, PB Rock, SR Muza, E Lammi, LG Moore, A Cymerman, CS Fulco. Slower rate of adductor pollicis muscle fatigue in women than in men. ***Med Sci Sports Exerc*** 29: S92, 1997.

Fulco CS, PB Rock, SR Muza, E Lammi, LG Moore, BA Beidleman, SF Lewis, A Cymerman. Adductor pollicis muscle fatigue in women during acute altitude exposure. ***Med Sci Sports Exerc*** 29: S135, 1997.

(14) Book chapter: Lewis, SF, CS Fulco. A new approach to studying muscle fatigue and factors affecting performance during dynamic exercise in humans. In: Exercise and Sport Sciences Reviews, Williams and Wilkins, Baltimore, 1998 (In press).

Manuscripts in preparation:

Fulco CS, PB Rock, SR Muza, E Lammi, LG Moore, A Cymerman, SF Lewis. Slower rate of adductor pollicis muscle fatigue in women than in men.

(15) Patent Copyright Applications: N/A

(16) Current Professional Address

Department of Health Sciences
Sargent College of Health and Rehabilitation Sciences
Boston University
635 Commonwealth Avenue
Boston, MA 02215

Current Home Address

PII Redacted

(17) Appraisal of the Associateship Programs

I found the NRC Associateship Program to be quite valuable. It provided me, as a senior scientist with many other responsibilities, with time to focus on further development of a research model capable of providing new and important information on both basic and applied aspects of human physical performance.

FINAL REPORT

(1) Date:	10/31/96
(2) Name (and ID number if known):	Dae Taek Lee
(3) Name and location of laboratory or center:	U.S. Army Research Institute of Environmental Medicine, Natick, MA
(4) Dates of tenure:	9/15/94 thru 11/15/96
(5) Title of research project:	Role of repeated reduction of core and skin temperatures on human cold acclimation.
(6) Research adviser's name:	Kent B. Pandolf, Ph.D.

(7) Are you on leave from a professional post? If so, list position or title and address of facility.

No.

(8) International posts held during tenure

N/A

(9) Programmatic travel during tenure. List location(s) and date(s)

N/A

(10) Scientific seminars, meetings, and/or consultations. List location(s) and date(s). List foreign meetings separately.

Lee, D.T., Toner, M.M., McArdle, W.D., Vrabas, I., & Pandolf, K.B. Human thermal responses to partial cold water immersion during rest and light exercise. American College of Sports Medicine, 43th annual meeting, Cincinnati, OH. 5/28/96 thru 6/2/96

Lee, D.T., Young, A.J., Bogart, J.E., & Pandolf, K.B. Finger vasodilatory responses in 4°C water. Experimental Biology '96, Washington, D.C. 4/13/96 thru 4/16/96

Young, A.J., Lee, D.T., Sawka, M.N., & Pandolf, K.B. Cold induced vasodilation in finger after cold water acclimation. Experimental Biology '96, Washington, D.C. 4/13/96 thru 4/16/96

American Society of Clinical Nutrition/American College of Sports Medicine, Post Graduate Course "Nutrition and Exercise for Performance and Health", Minneapolis, MN. 6/4/95

Lee, D.T., Haymes, E.M., Scott, B., & Trotter, D. Exercise duration and thermoregulatory responses after whole body pre-cooling. American College of Sports Medicine, 42th annual meeting, Minneapolis, MN. 5/30/95 thru 6/4/95

New England Regional Chapter of the American College of Sports Medicine, Boxborough, MA. 11/3/96

(11) Seminars or lectures delivered at universities and/or institutes. List location(s) and date(s).

"Effect of whole body pre-cooling on exercise duration and thermoregulatory responses during high-intensity running" at University of Buffalo, Buffalo, New York. 5/24/95.

(12) Meetings attended by specific invitation. List location(s) and date(s).

N/A

(13) Teaching, if any, as an associate.

N/A

(14) Work in progress.

Two scientific papers are under review processes for publication at the Journal of Applied Physiology. One additional manuscript will be submitted for publication at the Journal of Applied Physiology before the end of my tenure. Two abstracts are under preparation for presentation at the Experimental Biology '97 at New Orleans, Louisiana.

(15) Summary of research during tenure. Limit the summary to 100 words or less, single spaced, so that it may be entered into a permanent data bank in the Associateship Programs. Please do not used Greek letters or mathematical signs and symbols.

Repeated and simultaneous reduction of core and skin temperatures, induced by daily one hour immersions to shoulder level in 20 degree Celsius water for five weeks, produced an insulative type of cold acclimation including a more pronounced vasoconstriction in hands and fingers. A decrease in skin temperature without a decrease in core temperature did not induce cold acclimation, suggesting an increased heat flux was not, by itself, a stimulus for acclimation. The informations will assist improving cold tolerance and adaptability, and reducing cold injuries in soldiers.

(16) Publications and papers resulting from research as an associate. Provide complete citation(s), including author(s) name and initials, full name of journal, volume number, page number(s), and year of publication.

Lee, D.T., Young, A.J., Sawka, M.N., & Pandolf, K.B. Influence of whole body pre-cooling on cold-induced vasodilation. Journal of Applied Physiology, (in review)

Lee, D.T., Toner, M.M., McArdle, W.D., Vrabas, I.S., & Pandolf, K.B. Thermal and metabolic responses to cold-water immersion at knee, hip, and shoulder levels. Journal of Applied Physiology, (in review)

Young, A.J., & Lee, D.T. Aging and human cold tolerance. Experimental Aging Research, (in press)

(17) Patents applied for as a result of research as an associate.

N/A

(18) Future position and address or current forwarding address.

Forwarding address: [redacted]

PII Redacted

(19) Appraisal of the Associateship Programs. Comments on your program and its usefulness to you. Suggestions for improving the overall Associateship Programs.

It was a wonderful experience for two years, and I enjoyed every day as an Associate. I think that this program makes a smooth transition between school and real world possible, especially for those who are not native like me.

NATIONAL RESEARCH COUNCIL
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2101 CONSTITUTION AVE, NW, TJ 2114
WASHINGTON, DC 20418

FINAL REPORT

1.
Date: 9 May, 1997

2.
Fellow: James K. Wyatt, Ph.D.

3.
Laboratory: US Army Research Institute of Environmental Medicine, Natick, MA

4.
Dates of Tenure: 7/1/96 - 6/30/97

5.
Title of Research Proposal: The effects of oral melatonin and circadian- and sleep-related fluctuations on daytime simple and complex operations

6.
Research Advisor: Harris Lieberman, Ph.D.

7.
Leave: I am not on leave from a professional post.

8.
Professional Societies:
American Psychological Association
Sleep Research Society
American Sleep Disorders Association

9.
Professional Travel: Association of Professional Sleep Societies conference (June, 1997; San Francisco, CA)

10.
Seminars or Lectures:

Berkeley College of Music

1997 **Sleep and Circadian Rhythms: Implications for Good Health**
Guest Lecturer
Human Resources Department
Single Lecture

Brigham and Women's Hospital

1996 Course Co-Director, Continuing Medical Education Course entitled, "Diagnosis and Treatment of Sleep Disorders"

Circadian Rhythm Sleep Disorders
Lecture for BWH/HMS Continuing Medical Education Course
("Diagnosis and Treatment of Sleep Disorders")

Modulation of Neurobehavioral Functions by Circadian- and Sleep/Wake Dependent Processes
Endocrinology Research Conference
Single lecture

11.
Summary of Research:

During the past year, Dr. Lieberman and I have developed, validated, and implemented a comprehensive battery of neurobehavioral tests for use in long-term, circadian rhythms studies. Together with staff of the Brigham and Women's Hospital's Circadian, Neuroendocrine, and Sleep Disorders Section, we have studied 9 subjects on this protocol to date, and plan to study another 27 within the next 2 years. In addition, the test battery has been integrated into a flight-based protocol, for use by astronauts during NASA's 1997 Neurolab flight of the space shuttle Columbia. Effects of oral melatonin are not yet known, due to double-blind conditions.

12.
Research in Progress:

I will continue to work collaboratively with Dr. Lieberman (USARIEM) and Dr. Czeisler (Brigham and Women's Hospital / Harvard Medical School) to complete this human research protocol. We will continue to analyze the sleep (electroencephalogram, wrist actigraphy, sleep diary), circadian (core body temperature, plasma hormone values), and neurobehavioral measures (cognitive throughput, visual vigilance and attention, psychomotor tracking, short-term memory, subjective sleepiness and mood)

13.
Presentations at Scientific Meetings of Conferences:

I have been invited to speak during a discussion group on the topic of sleep and cognitive function, at the Association of Professional Sleep Societies conference this June.

14.
Publications:

To date, no publications have resulted from the research I conducted while an NRC Fellow. However, several papers have been published *during* the time of this fellowship, due to the inevitable time-lag between completion of past research and publication. These publications are the following:

Bell, I. R., Wyatt, J. K., Bootzin, R. R., & Schwartz, G. E. (1996). Slowed reaction time performance on a divided attention task in elderly with environmental chemical odor intolerance. International Journal of Neuroscience, 84, 127-134.

Richardson, G. S., Wyatt, J. K., Sullivan, J. P., Orav, E. J., Ward, A. E., Wolf, M. A., & Czeisler, C. A. (1996). Objective assessment of sleep and alertness in medical house-staff and the impact of protected time for sleep. Sleep, 19, 718-726.

Bell, I.R., Schwartz, G.E., Bootzin, R.R., & Wyatt, J.K. (1997). Time-dependent sensitization of heart rate and blood pressure over multiple laboratory sessions in elderly individuals with chemical odor intolerance. Archives of Environmental Health, 52, 6-17.

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15.

Patents: *No patents or copyrights applications have been filed during this NRC tenure*

16.

Future: I have accepted a postdoctoral fellowship at the following address, which is the laboratory with which Dr. Lieberman and I collaborated during the past year.

(forwarding address)
James K. Wyatt, Ph.D.
Circadian, Neuroendocrine and Sleep Disorders Section
Brigham and Women's Hospital
221 Longwood Avenue
Boston, MA 02115

17.

Comments: This NRC Fellowship Program has been very useful in my career development. It allowed me access to a top-level collaborative research team (between the USARIEM lab and the Brigham and Women's Hospital labs). The one-year commitment was also of benefit, in terms of flexibility -- just in case things did not work out for the best.